

Spectrum, antibiotic susceptibility and virulence factors of bacterial infections complicating severe acute pancreatitis

A.M. Israil¹, R. Palade², M.C. Chifiriuc¹, D. Vasile², M. Grigoriu², D. Voiculescu², D. Popa²

¹Cantacuzino Institute, Bucharest, Romania

²First Surgical Clinic of the University Emergency Hospital of Bucharest, Romania

Rezumat

Spectrul, sensibilitatea la antibiotice și factorii de virulență bacteriană în complicațiile infecțioase din pancreatita acută severă

Infecția secundară a țesutului necrotic pancreatic și peri-pancreatic reprezintă o complicație serioasă a pancreatitei acute severe (PAS), contribuind cu o rată semnificativă la morbiditate și mortalitate. Scopul prezentului studiu a fost de a determina spectrul etiologic al infecțiilor bacteriene, precum și profilurile de sensibilitate la antibiotice și de virulență ale tulpinilor izolate de la pacienți cu PAS. Un total de 19 cazuri de PAS au fost selectate randomizat din 153 de cazuri clinice de urgențe chirurgicale abdominale septice (vârsta 29-80 ani, sex 12 M/7F), internați în perioada 2009-2011 în Clinica I Chirurgică a Spitalului Universitar de Urgență București. 10 cazuri au prezentat culturi negative, deși opt dintre acestea au avut rezultate pozitive la bacterioscopie. Restul de nouă cazuri au prezentat culturi pozitive, din care au fost izolate și identificate 17 tulpini bacteriene. Infecția polimicrobiană a fost evidențiată la șase pacienți, iar etiologia dominantă a fost de bacili Gram negativi, urmată de bacterii anaerobe enterice, ceea ce atestă originea endogenă (colonică) a acestora. Testele de sensibilitate la antibiotice au confirmat eficiența *in vitro* a antibioticelor utilizate în tratamentul empiric pre-operator. Din cele 19 cazuri analizate, 14 au prezentat evoluție

favorabilă, iar cinci au avut sfârșit letal. Factorii dependenți de gazdă (sepsis, insuficiență multiplă de organe, alte comorbidități), cât și agresivitatea tulpinilor microbiene izolate, mediată de prezența diferiților factori implicați în aderență, toxigenitate și invazie, s-au dovedit a contribui la evoluția nefavorabilă, fatală a cazurilor de PAS. În ciuda tuturor controverselor teoretice, terapia antibiotică rămâne în prezent un mijloc terapeutic important pentru tratamentul PAS. Deși nu poate rezolva în proporție de 100% cazurile de infecția secundară a focarelor de necroză, asociat cu tratamentul chirurgical și celelalte mijloace medicale de terapie intensivă, tratamentul cu antibiotice poate schimba evoluția clinică în beneficiul pacienților, ducând la vindecarea acestora, într-un procent semnificativ de cazuri.

Cuvinte cheie: pancreatita acută severă, tratament empiric pre-operator, factori de virulență bacteriană

Abstract

Secondary infection of pancreatic necrotic tissue and peri-pancreatic fluid is a serious complication of acute pancreatitis resulting in significant morbidity and mortality. The aim of this study was to find out the spectrum of bacterial infections, their antibiotic susceptibility patterns and virulence features in patients with severe acute pancreatitis (SAP). A total of 19 patients with acute pancreatitis were consecutively selected from 153 clinical cases of septic abdominal surgical emergencies (age 29-80, 12 males, 7 females) admitted during 2009-2011, in the First Surgical Clinic of the University Emergency Hospital of Bucharest. All 19 SAP cases were submitted to pre-operative antibiotic empiric treatment. Ten cases were culture negative, in spite of the positive microscopy registered in eight

Corresponding author:

Mariana Carmen Chifiriuc MD
Cantacuzino Institute
Splaiul Independenței 103 Bucharest, Romania
E-mail: carmen_balotescu@yahoo.com

of them. The rest of nine cases were culture positive, 17 different bacterial strains being isolated and identified as belonging to eight aerobic and four anaerobic species. Polymicrobial infection was seen in six patients and the etiology was dominated by Gram-negative bacilli, followed by gut anaerobic bacteria, attesting their colonic origin. The susceptibility testing of the isolated strains confirmed in vitro in all cases the efficiency of the antibiotics that had been used in the empiric pre-operative treatment. Out of 19 cases submitted to pre-operative empiric treatment, 14 proved a favorable evolution and five a lethal outcome. The host depending factors (sepsis and other co-morbidities), as well as the aggressivity of the isolated microbial strains (mediated by the presence of different factors implicated in adherence, toxicity and invasion) were found to contribute to the unfavorable, even lethal clinical outcome of SAP cases. In spite of all theoretical controversies, the antibiotic therapy remains at present a very important therapeutic mean for the SAP treatment; although it cannot solve the septic necrotizing pancreatitis in 100% of cases, however, associated with the surgery and all other medical means of intensive therapy, the antibiotic treatment can influence the clinical evolution to the benefit and recovery of patients in a significant number of cases.

Key words: severe acute pancreatitis, virulence factors, antibiotic therapy

Introduction

The acute pancreatitis (AP) is a syndrome of glandular auto-digestion (1-3) of very different etiologies: i) common: biliary (including micro-lithiasis), alcoholism, hyperlipidemia, hypercalcemia, drugs and toxins, post-traumatic, postoperative, idiopathic; ii) uncommon: pancreatic neoplasia, vasculitis or iii) rare: viral or parasitic infections. In Romania, the highest frequency is reported for biliary, followed by alcoholic and metabolic AP.

AP may develop clinical forms with different degrees of severity, from simple acute pancreatitis (with acute edematous or interstitial pancreatitis), to mild cases (with focal or diffuse gland enlargement and small intrapancreatic fluid collection, accompanied by minimal organ dysfunction) counting for 70-90% of pancreatitis, with mortality < 1% and severe acute pancreatitis (SAP) with local complications as pancreatic necrosis, pseudocysts, abscesses and organ failure, developing especially in patients with low reactivity or septic shock, counting for 10-30% of AP cases with 10-40% mortality (4).

Most of the patients with AP are developing necrotizing pancreatitis (ANP) within the first 24 and virtually all within the first 72 h, following the onset of the clinical symptoms. The bacterial contamination of pancreatic necrosis, occurring in 40-70 % of cases (5), is the most common complication and most significant contributor to the clinical severity and

mortality rates of AP cases (16-66.7%) (6, 7).

The infectious etiology is monobacterial in 75% of cases, represented either by aerobic (*Escherichia coli*, *Proteus* sp., *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas aeruginosa* sp., *Enterococcus* sp., *Staphylococcus* sp., *Streptococcus mitis*, *Bacillus* spp.) or anaerobic (*Bacteroides* sp., *Veillonella* spp) strains (8-10).

Whereas the edematous pancreatitis requires only medical treatment (intensive therapy and parenteral administration of nutritive supplements), the SAP cases with high risk of infection, require surgical treatment, under pre- and post surgical antibiotic treatment. The timing of implementing the antibiotic and surgical treatment is crucial for the patient clinical evolution (11). This antibiotic prophylaxis is recommended to be started as soon as the diagnosis of ANP is made (by computer tomography), within the first days of disease, in order to avoid the infectious process.

The present paper reports the microbiological analysis of 19 clinical cases of SAP, treated in the First Surgical Clinic of the University Emergency Hospital of Bucharest, during 2009-2010, in the purpose to evidence the etiology implicated in the superinfected pancreatic necrotizing processes and to analyze the efficacy and safety of the empiric pre-operative antibiotic treatment, on the time point of infection and the patient outcome.

Material and Methods

Bacterial strains:

17 strains (13 aerobic and 4 anaerobic) isolated in nine clinical cases of SAP.

Standard strains:

Staphylococcus aureus ATCC no. 25923 reference strain used for CAMP test, *E. coli* ATCC no. 25922 and *Ps. aeruginosa* ATCC no. 27853 used as reference strains for antibiotic susceptibility testing.

Antibiotic disks:

Biomerieux disks: amoxicillin AMX (10 µg), amoxicillin + clavulanic acid AMC (20/10 µg), ceftazidim CAZ (30 µg), ceftriaxone CRO (30 µg), cefuroxime CXM (30 µg), ticarcillin TIC (75 µg), piperacillin PIP (100 µg), imipenem IMP (10 µg), ertapenem ERTA (10 µg), meropenem MERO (10 µg), gentamycin GM (10 µg), ciprofloxacin CIP (5 µg), norfloxacin NOR (10 µg), aztreonam AZT (30 µg), ticarcillin + clavulanic acid TIM (75/10 µg), ampicillin + sulbactam SAM (10/100 µg), cefoperazone + sulbactam (105 µg), cefepime FEP (30 µg).

Oxoid disks: penicillin P (10 U.I.), tigacyl TGC (75 µg), rifampicin RA (5 µg), vancomycin VA (30 µg), linezolid LZD (30 µg), clindamycin CC (2 µg).

Beckton Dickinson and Company disks: piperacillin + tazobactam TZP (100/10 µg).

Powder antibiotics: cefuroxim, piperacillin + tazobactam, ticarcillin, ertapenem.

The samples were intraoperatory collected by aspiration, using a syringe with fine needle, from intra-abdominal cavity, peritoneal space, necrotic pancreatic tissue, biliary ducts.

The aspirated products were injected in four different rubber stopped tubes containing different kinds of transport/preservation media: Cary Blair, nutrient broth, natrium thioglycolate and Schaedler medium.

The specimens were injected through the rubber stopper after the air having been expelled from the syringe and fine needle. Finally, the aspirate was used for preparation of two smears that were stained and microscopically examined by direct bacterioscopy (12).

Each sample was accompanied by the patient corresponding clinical data: name, surname, age, sex, diagnostic, pre-operative empiric treatment, immune status, immunosuppressive factors (radio-, chemo- therapy), type of specimens, lesion topographic localization, identification data of the medical staff implicated in the operatory process and specimen collection, data concerning samples collection (12).

Once arrived in the laboratory, the samples were inoculated simultaneously on: 5% sheep blood, MacConkey, Brucella blood agar supplemented with 5 µg/ml hemin and 1 µg/ml vitamin K and bile esculine agar plated media (12).

The 5% sheep blood and MacConkey agars were incubated in aerobic conditions at 35-37°C for 24 h, whereas the last two media were incubated in anaerobic conditions, in anaerobic bags with reduction substance, at 35-37°C, for 24-48 h (12).

All morphological types of colonies recovered from the nonselective aerobic and respectively anaerobic blood agar were sub-cultured to obtain pure cultures, further submitted to macroscopic (colony aspect, haemolysis) and microscopic (Gram staining affinity, morphology) examination and biochemical identification by help of conventional biochemical tests and API 20E, 20 NE and 20 A galleries. The reading and interpretation of biochemical aspects were done after 24-48 h incubation at 35-37°C (12).

The investigation of bacterial virulence potential was done by help of enzymatic tests, using the following specific media: 5% sheep blood agar (for alpha, beta haemolysins and CAMP-like factor), 15% casein agar (caseinase test), 3% gelatin agar (gelatinase test), 10% starch agar (amylase test), 1% porcine mucin in brain heart agar (mucinase test), DNA-agar (DN-ase test), 1% Tween agar (lipase test), 2.5% yolk agar (lecithinase test), Wagatsuma agar (Kanagawa enterotoxin test), 1% esculin iron salts (esculinase test), nutrient broth (slime test) and indole test (13,14).

All these tests were performed in aerobic/respectively anaerobic conditions depending of the identified strains. In case of anaerobic bacteria, the culture media were supplemented with hemin 5 µg/ml and vitamin K 1 µg/ml (12).

The antibiotic susceptibility testings were performed in concordance with CLSI 2011 standard recommendations for aerobic and respectively for anaerobic strains (15-16).

Results

Out of the 19 SAP cases (sex 12 M/7F, aged between 29-80 years

old) five were of alcoholic, three of biliary and 11 of idiopathic origin. The majority of cases (17) presented in their clinical history co-morbidities: hepato-biliary-pancreatic injuries, viral hepatitis type B, hepatic steatosis, cholecystitis lithiasis, pulmonary failure, chronic heart failure, acute and chronic renal failure, operated neoplasia, metabolic disturbances (dyslipidemia, diabetes mellitus, obesity), precarious general conditions, toxico-septic status and immunodeficiency. The respective AP cases evolved to infected hemorrhagic/necrotizing pancreatic area (Table 1).

It is to be pointed out that SAP cases were evidenced not only in aged persons over 60 years old, but also in young patients, in the twenties –fourties years of their life.

As late SAP complications, there were evidenced three cases with pancreatic abscesses and one with pseudocysts (Table 1).

In the 19 clinical cases, with different forms of SAP, specimens were collected from purulent areas of necrotic pancreatic tissue and from the retroperitoneal space. All cases were submitted to empiric pre-operative antibiotic treatment. Only nine cases were culture positive, whereas in the other ten, the cultures remained negative, in spite of the fact that the direct bacterioscopy indicated the presence of bacterial cells in eight of these last cases (Table 1).

The cases in which the presence of microbial agents was identified in the samples collected at the first surgical intervention were considered as primary infected pancreatitis (18).

When microbial agents i.e. one multidrug resistant (MDR)

Table 1. The association between different clinical parameters and the bacterial growth in SAP cases

Clinical parameters	Cases with positive culture	Cases with negative culture
Female	4	3
Male	5	7
Age	29-76	29-80
SAP-biliary	3	2
SAP alcoholic origin	2	3
SAP idiopathic	1	3
SAP postneoplastic	1	0
SAP with necrosis	5	2
Haemorrhagiae complications	2	3
Pseudocysts	1	0
Abscesses	2	2
Pulmonary failure	3	4
Chronic renal failure (CRF)	2	2
Acute renal failure (ARF)	1	3
Heart failure	1	2
Severe clinical condition	1	1
Multiple organ failure (MOF)	2	5
Septic shock	5	2
Co-morbidities	9	7
Immunodeficiency	3	2
No. of surgical interventions 1-3	between 1-3	between 1-6
Bacterial virulence factors	between 2-9	0
Clinical outcome	6	8
In-hospital mortality	3	2
Hospitalization days	21-59	14-110
Total no. of cases	9	10

Table 2. Clinical aspects of pancreatitis cases with positive cultures

Case no.	Diagnosis	Age	Sex	Comorbidities	Sepsis	Immunodepression	MOF	Isolated strains
2.	Alcoholic SAP	42	M	+	-	-	-	<i>E. coli</i>
3.	Haemorrhagic necrotizing SAP	61	F	+	+	-	-	<i>E. coli</i>
4.	Alcoholic necrotizing SAP	34	M	+	+	+	-	I. Gram-positive bacilli (OM) II. <i>Acinetobacter baumannii</i> MDR
5.	Suppurative necrotizing SAP	58	F	+	-	+	+ +	I. - II. <i>Citrobacter</i> sp. <i>Pseudomonas aeruginosa</i>
6.	I. Relapsing AP II. Pseudocysts	53	M	+	+	+	-	I. MSSA II. MRSA, <i>E. coli</i>
7.	Necrotizing SAP	29	F	+	-	-	-	<i>Bacteroides fragilis</i>
8.	Biliary necrotizing SAP	76	M	+	-	-	-	<i>Enterococcus</i> sp. <i>Actinomyces israelii</i>
9.	Suppurative necrotizing SAP	63	M	+	-	-	-	<i>E. coli</i> <i>Citrobacter</i> sp. <i>Bifidobacterium</i> sp.
10.	Colecisto-pancreatitis	69	F	-	+	-	+	<i>E. coli</i> MDR <i>K. ozaenae</i> <i>Bacteroides ovatus</i>

Legend: MOF - multiple organ failure, MDR - multiple drug resistance, I. - first surgical intervention, II. - second surgical intervention, AP - acute pancreatitis, SAP - severe acute pancreatitis, OM - optic microscopy

Acinetobacter baumannii, one MDR *E. coli* and one MRSA strain were isolated after surgical reintervention, the respective cases were considered as nosocomial infected pancreatitis. These infections were correlated with low reactivity (advanced age, immunodepressive state, long hospital stay in intensive care units) (Table 1).

In the nine culture positive cases (table 1), there were isolated 13 aerobic (5 *E. coli*, 2 *Citrobacter* spp., 1 *Klebsiella ozaenae*, 1 *Ps. aeruginosa*, 1 *Enterococcus* sp., 1 *Acinetobacter baumannii*, 1 MSSA and 1 MRSA) and 4 anaerobic (1 *Actinomyces israelii*, 1 *Bacteroides fragilis*, 1 *Bacteroides ovatus* and 1 *Bifidobacterium* sp.) strains. No fungal growth was obtained. Polymicrobial was observed in 5 and monomicrobial etiology in 4 cases (Table 2).

The evaluation of empiric pre-operative antibiotic treatment was made by testing in vitro the antibiotic susceptibility of the isolated bacterial strains. The enterobacterial strains were susceptible to imipenem, tigecycline, piperacillin + tazobactam, aminoglycosides and ampicillin+sulbactam, highly resistant to penicillins (amoxicillin, piperacillin, ticarcillin), amoxicillin +clavulanic acid and monobactams, and moderately resistant to 3rd cephalosporins and quinolones.

The anaerobic strains were susceptible to penicillin and beta-lactamase inhibitors associations (amoxicillin +clavulanic acid, ampicillin + sulbactam, piperacillin + tazobactam), imipenem, 2nd (cefoxitin, cefuroxim) and 3rd cephalosporins (cefotaxime), and resistant to monobactams (aztreonam).

In all nine culture positive cases, the antibiotics used in the empiric pre-operative treatment proved to be active in vitro against the isolated bacterial strains (Table 3). All patients submitted to broad-spectrum antibiotic prophylaxis received simultaneously nutritional support by naso-jejunal

feeding, rigorous fluid resuscitation, intensive supportive care (some of them in intensive care units) and management of pancreatic necrosis.

However, despite the in vitro efficiency of antibiotics used for prophylaxis, relapsing events occurred requiring 2-6 surgical interventions (exploratory laparotomy, necrosectomy, viscerolysis, debridement, drainage of the abscesses and peritoneal cavity, ascitis evacuation) and a re-escalation of the antibiotic treatment was used to stop the infectious process and the emergence of new septic centers and complications (hemorrhage, digestive fistula, purulent collections).

It must be pointed out that these SAP cases evolved in patients with low reactivity, multiple metabolic disturbances (dyslipidemia, hepatic steatosis, obesity), immunodeficiency state, multiple organ failure. Three of these cases with many co-morbidities deceased, whereas the other six had a favorable evolution, leaving the hospital after 21-59 hospitalization days. Tigecycline proved to be efficient in the postoperative treatment of a nosocomial infected pancreatitis with a multidrug resistant *Acinetobacter baumannii*, the patient recovering and leaving the hospital after 59 days.

Out of the ten culture negative cases, all submitted to empiric pre-operative treatment, the eight cases positive in microscopy proved the efficiency of the respective treatment pleading for the necessity of it in the SAP clinical cases. Among these last ten cases, eight evolved favorably, leaving the hospital after 14 to 110 hospitalization days, whereas two cases (with immunodeficiency, multiple organ failure and sepsis) had a fatal evolution.

Concerning the enzymatic equipment of the isolated aerobic and anaerobic strains possibly implicated in their virulence, there were evidenced up to 13 different enzymes,

Table 3. Bacteriological monitoring of the efficacy of empiric antibiotic therapy in SAP cases

		Empiric treatment	Isolated strains	Antibiotic susceptibility testing results	Treatment efficacy	Clinical outcome
1	Aerobic strains	Ampicillin+sulbactam	<i>E. coli</i>	SAM-S	E	R
2	Aerobic strains	Ertapenem Imipenem+ cilastatin Vancomycin	<i>E. coli</i>	ETP-S	E	R
3	Aerobic strains	I. Cefoperazone+sulbactam Metronidazole Vancomycin Tazobactam II. Tigecycline	I. Grampositive bacilli (OM) II. MDR <i>A.boumannii</i>	- Tigecycline (15 mm diameter)	- E	 R
4	Aerobic strains	I. Ampicillin Imipenem+ cilastatin Levofloxacin	I. Gram-positive cocci (OM) II. <i>Citrobacter</i> sp. <i>Pseudomonas aeruginosa</i>	- IMP-S IMP-S	- E E	 R
5	Aerobic strains	I. Imipenem+ cilastatin II. Imipenem+ cilastatin III. +Linezolid Imipenem+ cilastatin +Vancomycin	I. MSSA II. MRSA MDR <i>E. coli</i>	LZD-S VAN-S IMP-R, Imipenem-cilastatin -S	 E	 R
6	Facultative-ly/obligate anaerobic strains	Imipenem + cilastatin Teicoplanin Ciprofloxacin	<i>Bacteroides fragilis</i>	Imp-S	E	R
7	Facultative-ly/obligate	I. Cefoperazone+sulbactam Gentamycin II. Tazobactam+piperacillin Teicoplanin Ertapenem III. Imipenem + cilastatin	I. <i>Enterococcus</i> sp. <i>Actinomyces</i> sp.	Gen-S, SAM-S SAM-S	E	Deceased
8	Aerobic - anaerobic association anaerobic strains	I. Ceftriaxona Ertapenem II. Ertapenem III. Imipenem+cilastatin Colistin Vancomycin	<i>E. coli</i> <i>Citrobacter</i> sp., <i>Bifidobacterium</i> sp. <i>E. coli</i>	CRO-R ETP-R ETP-S ETP-S	Resistant Resistant E E	 R
9		I. Ampicillin+sulbactam +Gentamycin Cefoperazone+ Sulbactam Gentamycin Imipenem+ cilastatin Ertapenem II. Ciprofloxacin Ceftriaxona Tigecycline	I <i>E. coli</i> <i>K. ozenae</i> <i>Bacteroides ovatus</i>	GEN-S, IMP-S, CIP-S, TGY-S GEN-S, IMP-S, CIP-S, TGY-S GEN-S, IMP-S, CIP-S, TGY-S	Yes	Deceased (Septic shock) Chronic renal failure with haemodialysis Nitrogen retention)

R - recovered

E - efficient

among which the most frequent were: mucinase, caseinase and lipase. However, in the clinical cases with polymicrobial etiology, the number of the enzymatic virulence factors summarized up to 3-9 different factors/case, explaining the high aggressivity of the respective bacterial associations, expressed by their ability of multiplication, soluble factors secretion, invasivity and toxigenicity (Table 4). To the enzymatic equipment was added the ability of these strains to produce an extracellular exopolysaccharide evidenced by the slime test.

Discussion

Infection is a serious complication of acute pancreatitis and is the most common cause of death. It is determined most often by translocated enteric bacteria (18,19) and is commonly present in necrotizing pancreatitis. Although the most probable source of pancreatic infection seems to be the colon via bacterial translocation, the precise route of bacterial trans-mural migration is still unknown. Possible pathways are also via lymphatics and consecutive haematogenous spread, or transmurally to the pancreas (20).

Table 4. Virulence factors (VF) of bacterial strains isolated in SAP cases

		Species	Virulence factors (VF)														VF no.	Total no of VF/case
			Haemolysin	CAMP	Lipase	Lecithinase	Kanagawa haemolysin	Caseinase	Gelatinase	Mucinase	Esculinase	DNase	Amylase	Slime test	Indole production	Mannitol fermentation		
1.	Aerobic strains	<i>E. coli</i>									+			+	+		3	3
2.		<i>E. coli</i>												+	+		2	2
3.		<i>Acinetobacter boumannii</i>			+		+							+			3	3
4.		<i>Citrobacter sp.</i> <i>Pseudomonas aeruginosa</i>	+		+							+		+	+		5 5	6
5.		MSSA MRSA <i>E. coli</i>			+	+		+		+		+		+	+		7 7 2	9
6.	Anaerobic strains	<i>Bacteroides fragilis</i>			+			+				+					3	3
7.	Facultative /obligate anaerobic strains	<i>Enterococcus sp.</i> <i>Actinomyces israeli</i>	+							+	+						1 2	3
8.	Aerobic - anaerobic association	<i>E. coli</i>		+				+		+				+			3	6
		<i>Citrobacter sp.</i>						+		+							3	
		<i>Bifidobacterium sp.</i>						+	+	+	+						4	
9.		<i>E. coli</i> <i>K. ozanae</i>			+		+	+			+	+					5 4	9
		<i>Bacteroides ovatus</i>			+										+		2	

Concerning the bacteriology of the infected pancreatic necrosis, the positive cases revealed especially polymicrobial etiology (either associations of two or three aerobic strains, or aerobic-anaerobic strains associations). The above-mentioned results are different from those reported by other authors, stating that in the course of severe acute pancreatitis, cultures of infected pancreatic necrosis yielded monomicrobial flora in 60–87% of cases (21,22).

A preponderance of Gram negative aerobic bacteria suggesting an enteric origin was present (*Escherichia coli*, *Acinetobacter boumannii*, *Klebsiella* spp., *Citrobacter* sp., *Pseudomonas aeruginosa*), our data converging with other similar studies (21). In our case, Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus* sp.) and anaerobes were also found.

Although *Candida* species are recently reported in 21% of patients with infected pancreatic necrosis (21,22), no positive yeast culture was found among our analyzed cases.

Concerning the culture positive clinical cases submitted to empiric pre-operative treatment, the severity of the respective

SAP cases could equally be due to host (low reactivity, severe clinical status, sepsis, immunodeficiency, renal chronic failure with nitrate retention) and microbial (antibiotic resistance, enzymatic virulence factors, ability to produce a protective extracellular polysaccharide shell) related factors.

The microbial adherence to different substrata represents a sine qua non condition for the colonization and infection process. The ability of some of the tested strains to produce the so-called “slime” exo-polysaccharide could be an indicator for the adherence ability of the respective strains, for their resistance to phagocytosis process and other bactericidal factors (i.e. complement), for persistence on biotic and abiotic surfaces (in hospital on medical devices and in the external medium) (14).

Lipase and lecithinase, acting as pore-forming toxins could determine severe injuries of the cellular structures, such as pancreatic cell necrosis, favoring the bacterial invasivity process (23,24).

Other enzymes, like caseinase, are implicated in the protein hydrolysis having as consequence also severe injuries of

the pancreatic tissue, favoring the progression of the infectious process (25), whereas DNase, is inducing cellular DNA disintegration, generating severe injuries in the host cells (26).

Mucinase is an enzymatic complex able to split the mucin protector layer of the enteral lumen, favoring bacteria adherence to the enterocytes receptors. The resulted products from the mucin hydrolysis are covering the bacterial cells, protecting them from the gastric acidity and other molecules with anti-infectious activity (25).

Kanagawa factor (evidenced *in vitro* on Wagatsuma medium) is a haemolytic factor, with role of enterotoxin determining the pore formation at the enterocyte surface, the consequence being the hypersecretion of the enteral liquid (27).

The esculinase is responsible for esculine hydrolysis, resulting the esculetin, which could act as an iron chelator agent, providing iron even in limited conditions (28).

The amylase is responsible for polysaccharides degradation, ability conferring competitive nutritional advantages to the bacterial cells (13,14).

The implication of these enzymes in the severity of the studied cases was proved by the fact that in two cases with fatal evolution, the number of enzymes per case implicated in the virulence of the bacterial strains was high, summarizing six and respectively nine factors.

The survival of aerobic, anaerobic and aerobic-anaerobic associations of microbial agents in the necrotic pancreatic tissue (pancreatic sepsis) in spite of the intensive antibiotic therapy, could be explained by the microaerophilic conditions occurring in the necrotizing tissue, as result of a precarious vascularisation and adaptability of the respective bacterial cells to the local conditions. At the same time, it must be taken into account that for certain antibiotics the blood pancreas barrier could prevent the antibiotic from reaching the MIC (minimum inhibitory concentration) level into the infected necrotizing pancreatic tissue, whereas other antibiotics with an "efficacy factor" near the optimum (maximal value of 1) possess a high ability of penetration into the pancreatic tissue and of bacterial inhibition (29).

With special reference to the empiric pre-operative treatment, in cases of superinfected necrotizing pancreatitis, the "efficiency factor" of the selected antibiotics must be taken into account (30). This factor is signifying the ratio between the bacterial spectrum covered by a certain antibiotic panel and the penetration ability of the respective antibiotics, at least at MIC level, into the pancreatic tissue. At present the most recommended first-choice antibiotics are: carbapenems (i.e. imipenem including here also imipenem-cilastatin) and fluoroquinolone groups (ciprofloxacin, ofloxacin (30-33)). These antibiotics have been also used in the present study.

The timing of the surgical intervention by patients with SAP is still a controversial subject among the specialists, the literature indicating a very high morbidity and mortality, after PAS surgical treatment, in spite of implementing the empiric pre-operative antibiotic treatment associated with early enteral nutritional support, rigorous fluid resuscitation and close monitoring (34-37).

The literature indicates that simultaneously with an adequate pre-operative therapy, its duration is also very important; an empiric treatment must be used unchanged seven days and only if after seven days, the clinical symptoms are persistent, it is necessary to change the antibiotic treatment in concordance with the bacteriological testing (38-39).

In the present study, the empiric pre-operative treatment was performed in concordance with the last indications of the international guidelines (40). The treatment was implemented if necessary even 4-6 days before the first surgical intervention (in cases with severe evolution since the debut of the SAP process), constantly used for a period of 7-14 days and readjusted only in case of fever, unfavorable conditions, necessity of surgical reintervention or new indications provided by the bacteriological testing (re-escalation method) (40).

Out of the nine cases with positive cultures, in eight of them all isolated bacteria proved by antibiotic susceptibility testings *in vitro*, to be sensitive to at least one of the antibiotics used in the empiric pre-operative treatment (Table 3), whereas in one case (no. 8), where an aerobic-anaerobic association was present, *Citrobacter* and *Bifidobacterium* strains proved to be susceptible to ertapenem, but *E. coli* strain resistant to both antibiotics (ceftriaxone + ertapenem); when these two anti-microbials were de-escalated and substituted with imipenem-cilastatin, the clinical outcome improved evidently and the patient recovered.

Simultaneously with the empiric treatment, there were applied also: an intensive complex therapy for restoring patient homeostasis, parenteral (per nasal /per jejunum tube) nutritional support in the purpose to restore the normal functions of the enteral mucosa (40).

Concerning this last aspect it must be taken into account that the enteral feeding, implemented as soon as possible, is decreasing the risk of bacterial translocation in patient with PAS, repairs the mucosal damage, of fasting, favoring the recovery of the epithelial integrity and enteral ecology, helping the maintenance of the gut barrier function (41-43).

The corner stones in the management of the severe abdominal infections remain: the identification of the source of microbial agent and the adequate antibiotic therapy, for both objectives, the bacteriological laboratory playing an important role.

In the present study, in spite of the fact that the results of the susceptibility testing have proved the efficacy of the respective antibiotics *in vivo*, however out of 19 cases submitted to the pre-operative treatment, five cases had a fatal evolution. These five patients proved low reactivity, multiple co-morbidities and immunodeficiency state, elements favoring the multiplication and high aggressivity of the microbial agents. These aspects are pointing out once more again the importance of antibiotic therapy in prophylactic purpose. It means not only to prevent the superinfection of the necrotizing pancreatic tissue, but also to prevent microbial dissemination and colonization of other tissues (i.e. pneumonia, purulent pleuresia, hepatic abscesses), especially in patients with multiple organ failure (44, 45).

There are authors (43) pleading for the unsustainability of

the empiric antibiotic pre-operative treatment in SAP, their opinion being based on one side on the inability of the respective antibiotics to penetrate and reach the necessary MIC level in the necrotic pancreatic and peripancreatic tissue, and on the other side based on the aspect that early and extensive usage of antibiotics in patients with critical state can determine certain changes in the microbial flora of the host organism by increasing and finally by re-extending the antibiotic resistance within the hospital to other patients. However these authors are adding that: "it is very difficult to prevent the clinicians not to implement prophylactic empiric antibiotic treatment to the patients".

Therefore, in spite of so much controversial moderate (second-rate) results, the benefits of the empiric pre-operative treatments in SAP cases could not be categorically rejected.

A beneficial prophylactic effect with antibiotics such as metronidazole, imipenem, and third-generation cephalosporins was observed in different studies. In 2010, Villatoro et al. (40) showed that although they cannot confirm benefit from the use of prophylactic antibiotics, "consistent trends towards a beneficial effect nevertheless remain". These authors recommended in case of infected necrosis antibiotic be given before surgery, carbapenems, being preferred to other antibiotics, the treatment lasting 2-4 weeks.

The most recent medical literature (46) provides convincing evidence that early antibiotic prophylaxis is beneficial and should therefore form part of the standard treatment of patients with SAP.

In our study, out of 19 cases of SAP submitted simultaneously to empiric pre-operative antibiotic, surgical treatment and intensive therapy treatment, in spite of five deceased cases, however 14 cases had a favorable outcome recovering and leaving the hospital after 20-110 days of hospitalization.

The selection of antibiotic therapy must be done taking into account the nature of the intra-abdominal infectious points, the type of infection, the severity of lesions ascertained intraoperatively, host reactivity and by using the "de-escalation" method.

Recent data of the literature are pointing out that the threat of microbial resistance has been identified as one of the major difficulty in managing the complicated intra-abdominal infections. The choice of empiric pre-operative antibiotic treatment can have a significant impact on the patient outcome when resistant microorganisms are involved. Taking into account these considerations, it is highly recommended that the sets of antibiotics included in the last international guideline for empiric treatments must be permanently controlled (by laboratory testing) for their efficacy on the bacterial strains circulating in a certain geographical area, in the purpose the emergence of resistant strains be promptly identified and the respective empiric antibiotic pre-operative treatment be readjusted to the new local conditions. This desideratum can be fulfilled only by close collaboration between the clinicians and the laboratory of bacteriology.

Conclusions

1. Out of 153 clinical cases of intra-abdominal surgical emergencies, hospitalized in the First Surgical Clinic of the University Emergency Hospital of Bucharest, during 2009-2010, 19 randomized cases (12%) were diagnosed with severe acute pancreatitis.
2. Out of the 19 SAP consecutive cases, nine exhibited positive cultures, the etiology being dominated by Gram-negative bacilli, attesting their colonic origin, but Gram-positive cocci and anaerobic bacilli were also isolated.
3. The *in vitro* antibiotic susceptibility testing of the isolated strains confirmed in all cases the efficiency of the antibiotics used in the empiric pre-operative treatment.
4. The host depending factors (sepsis and other comorbidities), as well as the aggressivity of the isolated microbial strains (mediated by the presence of different factors implicated in adherence, toxicity and invasion) proved to contribute to the unfavorable, even lethal clinical outcome of SAP cases.
5. In spite of all theoretical controversies, the antibiotic therapy remains at present an important therapeutic mean for the SAP treatment; although it cannot solve the septic necrotizing pancreatitis in 100% of cases, however associated with surgery or other medical means of intensive therapy can change, the clinical evolution to the benefit and recovery of the patients, in a significant number of these cases.
6. Concerning the resistance to antibiotics included in the empiric pre-operative treatment, it becomes necessary the periodic testing of their *in vitro* efficacy on the isolated bacterial strains circulating in a certain geographical area be performed and consequently, the respective set of antibiotics be quickly readjusted to the new epidemiological conditions.

Acknowledgements

The authors kindly acknowledge the technical assistance of Mrs. Geta Șoricilă. The present work was supported by the Ministry of Education and Research Grant CNMP Project 42-150/2008-2011.

Selected References

1. Marincaș M, Brătucu E, Tobă M, Cirimbei C, Păun L. Surgical approach in acute necrotizing pancreatitis. *Chirurgia (Bucur)*. 2006;101(3):237-47. [Article in Romanian]
2. Palade RS. Patologia chirurgicală a pancreasului. În: *Manual de Chirurgie Generală*, ed. II-a, vol. 2. București: Ed. All; 2008. p. 151-207.
3. Popescu I. Management of acute severe pancreatitis. *Chirurgia (Bucur)*. 2006;101(3):225-8. [Article in Romanian]
4. Stevens T, Parsi MA, Walsh RM. Acute pancreatitis: problems

- in adherence to guidelines. *Cleve Clin J Med*. 2009;76(12):697-704.
5. Toouli J, Brooke-Smith M, Bassi C, Carr-Locke D, Telford J, Freeny P, et al. Guidelines for the management of acute pancreatitis. *J Gastroenterol Hepatol*. 2002;17 Suppl:S15-39.
 6. Gloor B, Müller CA, Worni M, Stahel PF, Redaelli C, Uhl W, et al. Pancreatic infection in severe pancreatitis: the role of fungus and multiresistant organisms. *Arch Surg*. 2001;136(5):592-6.
 7. Diculescu M, Ciocirlan Mirela, Cicirlan M, Stoicescu Adriana, Stanescu D, Ciprut T. Acute pancreatitis and its frequent complications. Acute pseudocysts and fluid collections in: *Intra-abdominal Sepsis Unresolved Issues*. edited by C.Vasilescu and Hans Berger. București: Editura Acad. Romane; 2006. p. 203-211.
 8. Sartelli M. A focus on intra-abdominal infections. Review. *World Journal of Emergency Surgery*, 2010. 5:9 on line <http://www.wjes.org/content/5/1/9>
 9. Baron TH, Morgan DE. Acute necrotizing pancreatitis. *N Engl J Med*. 1999;340(18):1412-7. Erratum in: *N Engl J Med* 1999; 341(6):460.
 10. Berger HG. Sepsis in necrotizing pancreatitis-definition, pathophysiology and prognostic in Intra-abdominal Sepsis. *Proceedings of the First International Humboldt Workshop on Surgical Research*, Bucharest; 2005. p. 141-142.
 11. Götzinger P, Wamser P, Exner R, Schwanzer E, Jakesz R, Függer R, et al. Surgical treatment of severe acute pancreatitis: timing of operation is crucial for survival. *Surg Infect (Larchmt)*. 2003; 4(2):205-11.
 12. Israil AM, Delcaru C, Palade RS, Chifiriuc C, Iordache C, Vasile D, et al. Bacteriological aspects implicated in abdominal surgical emergencies. *Chirurgia (Bucur)*. 2010;105(6):779-87.
 13. Israil A, Balotescu C, Bucurenci N, Năcescu N, Cedru C, Popa C, et al. Factors associated with virulence and survival in environmental and clinical isolates of *Vibrio cholerae* O1 and non O1 in Romania. *Roum Arch Microbiol Immunol*. 2003; 62(3-4):155-77.
 14. Panus Emilia, Chifiriuc Mariana Carmen, Banu Otilia, Mitache Magda, Bleotu Coralia, Rosoiu Natalia, Lazăr Veronica. Comparative study of resistance and virulence markers in *Escherichia coli* strains isolated from hospital surfaces, clinical specimens and drinking/ marine waters. *BRIAC*, 2011. 1: 24-30
 15. CLSI Performance Standards for Antimicrobial Susceptibility Testings Twenty First Informational Supplement 2011, vol.30, no. 1 and 15. p. ???
 16. CLSI Methods for Antimicrobial Susceptibility Testings of Anaerobic Bacteria; Approved Standard Seventh Edition 2007, vol.27, nr.2. p. ???
 17. Kollef M. Appropriate empirical antibacterial therapy for nosocomial infections: getting it right the first time. *Drugs*. 2003;63(20):2157-68.
 18. Runkel NS, Moody FG, Smith GS, Rodriguez LF, LaRocco MT, Miller TA. The role of the gut in the development of sepsis in acute pancreatitis. *J Surg Res*. 1991;51(1):18-23.
 19. Cicalese L, Sahai A, Sileri P, Rastellini C, Subbotin V, Ford H, et al. Acute pancreatitis and bacterial translocation. *Dig Dis Sci*. 2001;46(5):1127-32.
 20. Bakoyiannis A, Delis S, Kusske CD, Rongione AM, Reber HA. Cytokines and acute pancreatitis. *Gastroenterology*. 1996;110(2):639-42.
 21. Medich DS, Lee TK, Melhem MF, Rowe MI, Schraut WH, Lee KK. Pathogenesis of pancreatic sepsis. *Am J Surg*. 1993; 165(1):46-50; discussion 51-2.
 22. Berzin TM, Morteale KJ, Banks PA. The management of suspected pancreatic sepsis. *Gastroenterol Clin North Am*. 2006;35(2):393-407.
 23. Bhakdi S, Weller U, Walev I, Martin E, Jonas D, Palmer M. A guide to the use of pore-forming toxins for controlled permeabilization of cell membranes. *Med Microbiol Immunol*. 1993;182(4):167-75.
 24. Lee SH, Choi JI, Park SJ, Lee SY, Park BC. Display of bacterial lipase on the *Escherichia coli* cell surface by using FadL as an anchoring motif and use of the enzyme in enantioselective biocatalysis. *Appl Environ Microbiol*. 2004;70(9):5074-80.
 25. Swamy KH, Goldberg AL. Subcellular distribution of various proteases in *Escherichia coli*. *J Bacteriol*. 1982;149(3):1027-33.
 26. Buchanan JT, Simpson AJ, Aziz RK, Liu GY, Kristian SA, Kotb M, et al. DNase expression allows the pathogen group A *Streptococcus* to escape killing in neutrophil extracellular traps. *Curr Biol*. 2006;16(4):396-400.
 27. Ross CA. Mucinase activity of intestinal organisms. *J Pathol Bacteriol*. 1959;77(2):642-4.
 28. Coulanges V, André P, Vidon DJ. Esculetin antagonizes iron-chelating agents and increases the virulence of *Listeria monocytogenes*. *Res Microbiol*. 1996;147(9):677-85.
 29. Uomo G. Antibiotic treatment in acute pancreatitis. *Rocz Akad Med Białymst*. 2005;50:116-21.
 30. Sharma VK, Houlden CW. Prophylactic antibiotic administration reduces sepsis and mortality in acute necrotizing pancreatitis, a meta-analysis. *Pancreas*. 2001. p. 22-28.
 31. Traverso LW. Infections complicating severe pancreatitis. *Infect Dis Clin North Am*. 1992;6(3):601-11.
 32. Maves RC, Halsey ES. Anaerobic coverage in intra-abdominal and biliary infections. *Surg Infect (Larchmt)*. 2011;12(2):149. Comment on *Surg Infect (Larchmt)*. 2010;11(1):79-109.
 33. Mazuski JE. Antimicrobial treatment for intra-abdominal infections. *Expert Opin Pharmacother*. 2007;8(17):2933-45.
 34. Dellinger EP, Tellado JM, Soto NE, Ashley SW, Barie PS, Dugernier T, et al. Early antibiotic treatment for severe acute necrotizing pancreatitis: a randomized, double-blind, placebo-controlled study. *Ann Surg*. 2007;245(5):674-83.
 35. Pezzilli R. Antibiotic prophylaxis in acute necrotizing pancreatitis: yes or no? *JOP*. 2004;5(3):161-4.
 36. Popovici Raluca, Baldea Corina, Vasile P.GH., Vilceanu Ioana. Management Problems in Therapeutic Conduct of Acute pancreatitis of biliary Etiology; International Symposia Risk Factors for Environmental and Food Safety & Natural resources and Sustainable Development. Faculty of Environmental Protection, 2009. Nov. 6-7, Oradea, 1624-1631.
 37. Oláh A, Belágyi T, Bartek P, Pohárnok L, Romics L Jr. Alternative treatment modalities of infected pancreatic necrosis. *Hepatogastroenterology*. 2006;53(70):603-7.
 38. Laterre PF, Colardyn F, Delmée M, De Waele J, Legrand JC, Van Eldere J, et al. Antimicrobial therapy for intra-abdominal infections: guidelines from the Infectious Disease Advisory Board (IDAB). *Acta Chir Belg*. 2006;106(1):2-21.
 39. Eckmann C, Dryden M, Montravers P, Kozlov R, Sganga G. Antimicrobial treatment of "complicated" intra-abdominal infections and the new IDSA guidelines ? a commentary and an alternative European approach according to clinical definitions. *Eur J Med Res*. 2011;16(3):115-26.
 40. Villatoro E, Mulla M, Larvin M. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis. *Cochrane Database Syst Rev*. 2010;(5):CD002941.
 41. Blot S, De Waele JJ. Critical issues in the clinical management of complicated intra-abdominal infections. *Drugs*. 2005;65(12):

- 1611-20.
42. Bodmann KF; und die Expertenkommission der Infektliga. Complicated intra-abdominal infections: pathogens, resistance. Recommendations of the Infektliga on antibiotic therapy. *Chirurg*. 2010;81(1):38-49. [Article in German]
43. Noor MT, Radhakrishna Y, Kochhar R, Ray P, Wig JD, Sinha SK, Singh K. Bacteriology of infection in severe acute pancreatitis. *JOP*. 2011;12(1):19-25.
44. Schneider L, Büchler MW, Werner J. Acute pancreatitis with an emphasis on infection. *Infect Dis Clin North Am*. 2010; 24(4):921-41, viii.
45. Dellinger EP, Tellado JM, Soto NE, Ashley SW, Barie PS, Dugernier T, et al. Early antibiotic treatment for severe acute necrotizing pancreatitis: a randomized, double-blind, placebo-controlled study. *Ann Surg*. 2007;245(5):674-83.
46. Gloor B, Müller CA, Worni M, Stahel PF, Redaelli C, Uhl W, et al. Pancreatic infection in severe pancreatitis: the role of fungus and multiresistant organisms. *Arch Surg*. 2001;136(5): 592-6.