OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE www.BiointerfaceResearch.com ISSN 2069-5837

Volume 1, Issue 6, 2011, 209-220

Received: 11.11.2011 / Accepted: 10.12.2011 / Published on-line: 15.12.2011

Comparative analysis of disk diffusion and liquid medium microdillution methods for testing the antibiotic susceptibility patterns of anaerobic bacterial strains isolated from intra-abdominal infections

Mariana Carmen Chifiriuc [1]*, Radu Palade[2], Anca Michaela Israil[3]

ABSTRACT

The antibiotic resistance aspects concerning the bacterial strains isolated from intra-abdominal infections signify at present a major problem of therapy. The empiric pre-operatory antimicrobial therapy plays a key role in the management and course of the intra-abdominal infections, an inappropriate therapy resulting in a poor outcome of the clinical cases and an increase of bacterial resistance. The purpose of the present paper was to compare the results of the antibiotic susceptibility of some selected anaerobic strains to certain antibiotics used in the empiric therapy of intra-abdominal infections, achieved by two different methods, in order to select for the current practice the most reliable, simple and rapid one. We have found a good correlation between the results obtained by the standard, Brucella broth microdilution method recommended by CLSI and the disk diffusion method (recommended by Bailey and Scott, 2002), for all tested antibiotics, demonstrating the possibility to use this last simplified method as an alternative to the laborious and time-consuming dilution method, for the routine testing of the antibiotic susceptibility of anaerobic stranis isolated in severe infections.

Keywords: *anaerobic strains, intra-abdominal infections, antibiotic susceptibility patterns, breakpoint values, cut-off*

1. Introduction

Intra-abdominal infections often leading to emergency surgery exhibit very different clinical forms from acute appendicitis, gangrenous appendicitis, diverticulitis up to severe cases of bleeding perforated ulcer, cholecystitis with secondary peritonitis, liver abscesses, infected necrotizing severe acute pancreatitis, infected abdominal tumors etc., a lot of them representing an important cause of morbidity and being frequently associated with poor prognosis in high risk patients. In low-risk cases (involving a single organ), infectious agents are community bacterial strains, whereas in high risk cases (infections complicated by loss of integrity of the gastrointestinal tract) favored by the patient poor conditions, with high morbidity and mortality, there are frequently implicated unexpected pathogenic infectious agents and nosocomial strains. The patient's poor outcomes are associated

[1] Faculty of Biology, Microbiology Immunology Department, Ale. Portocalelor, 60101 Bucharest, Romania
*Corresponding author e-mail address: ***carmen_balotescu@yahoo.com***
[2] First Surgical Clinic of the Universitary Emergency Hospital of Bucharest, Spl. Independentei No.169 Sector 5, Bucharest, Romania
[3] National Institute for Research and Development in Microbiology and Immunology Cantacuzino, Apl. Independentei 103, Bucharest, Romania

with the severity of illness, health care –acquired infection, source control failure and inadequate empiric antibiotic therapy. The literature is pointing out that at present " a well defined evidence – recommendations for these clinical cases are partially missing because of the limited number of randomized-control led trials [1].The empiric pre-operatory antibiotic treatment plays a key role in the subsequent clinical outcome of the patients. A correct therapeutic approach requires an adequate empirical therapy with large spectrum antimicrobial agents. An empiric therapy can be considered efficient only if all isolates proved to be sensitive to at least one of the administered antibiotics. As soon as the etiological agent was isolated and the antibiotic susceptibility pattern was established, the empirical therapy must be adjusted in concordance with the laboratory results (re-escalation method). The empiric treatment not submitted to clinical and microbiological reevaluation can turn into a high percentage of failures (49%) in episodes of bacteremia of unknown etiology. Un-effective antibiotic treatment could lead to an inappropriately poor clinical evolution and death, antibiotic resistance, prolonged hospitalization, surgical re-interventions and high costs. Taking into account that the antimicrobial resistance is considered at present a major difficulty in managing the complicated intra-abdominal infections on one side and the aspect that the majority of the clinical laboratories do not routinely determine the species of anaerobic microorganisms and do not test the susceptibility of these strains because of the technical difficulties, the purpose of the present work was to test by comparison the efficiency of two different methods for the assessment of the antibiotic susceptibility in anaerobic bacterial strains belonging to *Bifidobacterium, Actinomyces* and *Bacteroides* species, randomly isolated from complicated intra-abdominal infections, in order to select the most reliable, simple and rapid assay for testing the antibiotic susceptibility in anaerobic strains. *Bifidobacterium* spp. non-sporulating, gram-positive anaerobic bacilli that could grow also in microaerophilic conditions are constituting part of the oral [2], respiratory and enteral human microbiota [3]. During the last two decades, the literature has cited more and more frequently, clinical cases in which the *Bifidobacterium* spp. strains are isolated from anaerobic intra-abdominal severe infections [4-10]. *Bacteroides* infections can develop in all body sites, including the CNS, the head, the neck, the chest, the abdomen, the pelvis, the skin, and the soft tissues. Inadequate therapy against these anaerobic bacteria may lead to clinical failure [11, 12]. Because of their fastidiousness, they are difficult to be isolated and consequently are often overlooked [13]. Treatment is complicated by three factors: slow growth, increasing resistance to antimicrobial agents and the polymicrobial synergistic nature of the infection [14]. *B. fragilis* predominates in endogenous intra-abdominal infections. Enterotoxigenic *B. fragilis* (ETBF) is also a potential cause of diarrhea [15]. Abdominal actinomycosis is the most frequently preceded by surgery for acute appendicitis, perforated ulcer, or gallbladder inflammation. The incidence of the abdominal-pelvic form of actinomycosis has increased over the past 10 years and could be the result of prolonged use of intra-uterine device (IUD) [16]. The clinical spectrum and the management of actinomycosis have dramatically changed, so have the therapeutic considerations [17].

2. Experimental section

2.1. Microbial strains: 5 *Bacteroides* sp., 5 *Actinomyces* sp. and 5 *Bifidobacterium* sp. were isolated from patients submitted to surgery for intra-abdominal emergencies, during 2009-2011 in the First Surgical Clinic of the University Hospital of Bucharest.

2.2. Type of specimens: liquid from intra-abdominal cavity, peritoneal space, ascite liquid, gallbladder and billiary duct content, duodenal liquid, ceccal and appendix content, pancreatic necrotic tissue.

2.3. Antibiotic disks: cefoxitin FOX (30 μg), cefotaxim CTX (30 μg), ertapenem ETP (10 μg), Piperacillin plus tazobactam TZP (100 μg /10 μg) and tigecycline TGC (15 μg).

2.4. Media: Brucella blood agar supplemented with vitamin K and hemin.

2.5. Other materials: anaerobic bags, exicator.

2.6. Methods

2.6.1. Samples collection: the samples were collected intraoperatory by aspiration with syringe and inoculated through rubber stopper into transport (thioglycolate and Schaedler) media; simultaneously two smears were done from each specimen for direct bacterioscopy.

2.6.2. Cultivation: the samples were inoculated on anaerobic (regenerated thyoglycolate and blood-Brucella agar with 5μl/ml hemin and 1 μg/ml vitamin K) media and thereafter the media were incubated at 37 ^{0} C for 48-72 hrs until 4 weeks in anaerobic bags with catalyst substance and kept in an exicator.

2.6.3. Strains identification: for strains identification, the macroscopic and microscopic (Gram stained smears) examination was done and thereafter the biochemical identification was performed by conventional phenotypic tests and API 20 A microgalleries.

2.6.4. Antibiotic susceptibility assays

2.6.4.1. Disk diffusion method for testing the antibiotic susceptibility of the isolated anaerobic strains was performed on Brucella blood agar (supplemented with hemin 5μg/ml and vitamin K 1 μg/ml) by disk diffusion method adapted to anaerobic conditions, i.e.: a portion of one colony of the tested anaerobic strain was transferred to Brucella blood agar plate. The Petri plate was streaked several times with this strain to produce a heavy lawn of bacterial growth. Thereafter the antibiotic disks were placed at 30 mm distance on the surface of the respective Petri plates. A quadrant of a plate was streaked only with the tested strain (without any antibiotic disk being added) and used as control of bacterial growth. All plates were incubated anaerobically for 48 hrs at 35 ^{0} C [18]. Only if the above-mentioned control exhibited bacterial growth, the inhibition areas of the bacterial growth around the disks were taken into consideration, i.e. an area of 10 mm or less was interpreted as resistance, whereas an area greater than 10 mm as susceptibility [3].

2.6.4.2. MIC assay by broth microdilution method for testing the antibiotic susceptibility of anaerobic strains. The 10x concentrated antibiotic stock solutions were used to obtain two-fold serial dilutions in a total volume of 10 ml of Brucella broth supplemented with hemin (5 μg/mL) and vitamin K1 (1 μg/mL) and lysed horse blood (5%). For each antibiotic, the scale of serial dilutions was performed in accordance with CLSI recommendations (2010) [19]. Thereafter, an amount of 495 μl of each antibiotic dilution was distributed in 96-well plates. Two well rows were used as positive (sterile broth and microbial inoculum) and respectively negative (sterile broth) controls. Also, an inoculated microdillution tray from each batch prepared in –house was incubated overnight to confirm sterility. Simultaneously, a 0.5 MacFarland standardized inoculum (containing approximately $1x10^8$ CFU/mL) was prepared for each strain, by suspending bacterial colonies in saline solution. Within 15 minutes after the preparation of the standardized microbial inoculum, each well was inoculated with 5 μl of microbial suspension, reaching a final density of $1x10^6$ CFU/mL in each well. The inoculated plates were sealed in plastic bags and immediately placed for incubation for 46-48 hours, at 35-37^{o}C in anaerobic conditions, using an exicator. After the

incubation period, the wells buttom was examined using a mirror, starting with the positive (with visible turbidity proving microbial growth) and negative (clear, sterile medium) control wells. The MIC value was determined as the lowest concentration at which no growth or the most significant reduction of the growth was observed.

2.4.6.3. Whonet 5.5 scatter plot data analysis. The growth inhibition zone diameters (mm) as well as the MIC values (µg/ml) were registered in Whonet 5.5 file and submitted to Data analysis by scatter plot option, which established for each antibiotic the concordance curves between MIC and zone inhibition diameter values, the breakpoints being also given for the respective strains.

2.4.6.4. Assessment of the epidemiological cut-offs of the bacterial strains. Determination of CMI breakpoints has an important clinical significance, defining the clinical antibiotic susceptibility degrees: susceptible, intermediate, resistant (S<= x, I=x-y, R>=y). Beside these values, another quantitative parameter of epidemiological significance is the cut-off value, required to make the difference between the bacterial population belonging to the wild phenotype (bacterial strains without resistance markers) on one side and the circulating bacterial population belonging to the same species, with acquired resistance phenotypes to a certain antibiotic, on the other side. By a simplified method, the established cut-off value, corresponding to the susceptibility of the wild type bacterial population, is considered the equivalent to the MIC breakpoint defining the S clinical category for a certain antibiotic (www.eucast.org). A more accurate method to establish the cut-off values requires many steps: i) the use of a more complicated dilution scheme with dilutions steps lower than the classical serial two fold dilutions, as in E-Test; ii) a mathematical processing of the achieved data and namely: the MIC lowest value is used in a formula, in the purpose to convert this value into a theoretical value of inhibition diameter considered as equivalent of the inhibition area (ZE): $ZE=50-(\log_2 MIC+10)$; statistical processing of data achieved on a significant number of strains by using a special software for data processing (NRI) (Bioscand AB, Taby, Sweeden, International Patent Application WO 02/083935 A1). By this method, the significance of the achieved ZE value is that the strains of wild type susceptible to a certain antibiotic must display a larger or an equal inhibition area with the ZE value obtained by the above-mentioned formula. In this context, the wild bacterial population is defined as the susceptibility limit expressed by the number of standard deviations (SD) from the middle value. For example, a number of two standard deviations (2SD) must be considered as including 97,7% of the sensitive strains, 2,5 SD -99,4 sensitive strains, and 3SD-99,9% of the strains. The values of the inhibition zone diameters obtained for the circulating strains are often located under the ZE value. In the graphic representations of the cut-off values, the nearer to the ZE line the values of a circulating phenotype are placed, the higher level of susceptibility these strains are displaying.

3. Results section

The choice of the pre-operatory and post-operatory antibiotic therapy in infections implicated in intra-abdominal emergence surgery must take into account the fact that anaerobic bacterial strains are isolated in 80% of cases of complicated appendicitis and in a high number of intra abdominal infections (20). Recent data are pointing out that the emergence of microbial resistance in aerobic, as well as in anaerobic strains is one of the major difficulty in managing the complicated intra-abdominal infections, so that the choice of empiric pre-operatory antibiotic treatment can have a significant impact on the patient outcome when resistant microorganism are involved. As already

mentioned the empiric pre-operatory antibiotic treatment plays a key role in the subsequent clinical outcome of the patients.

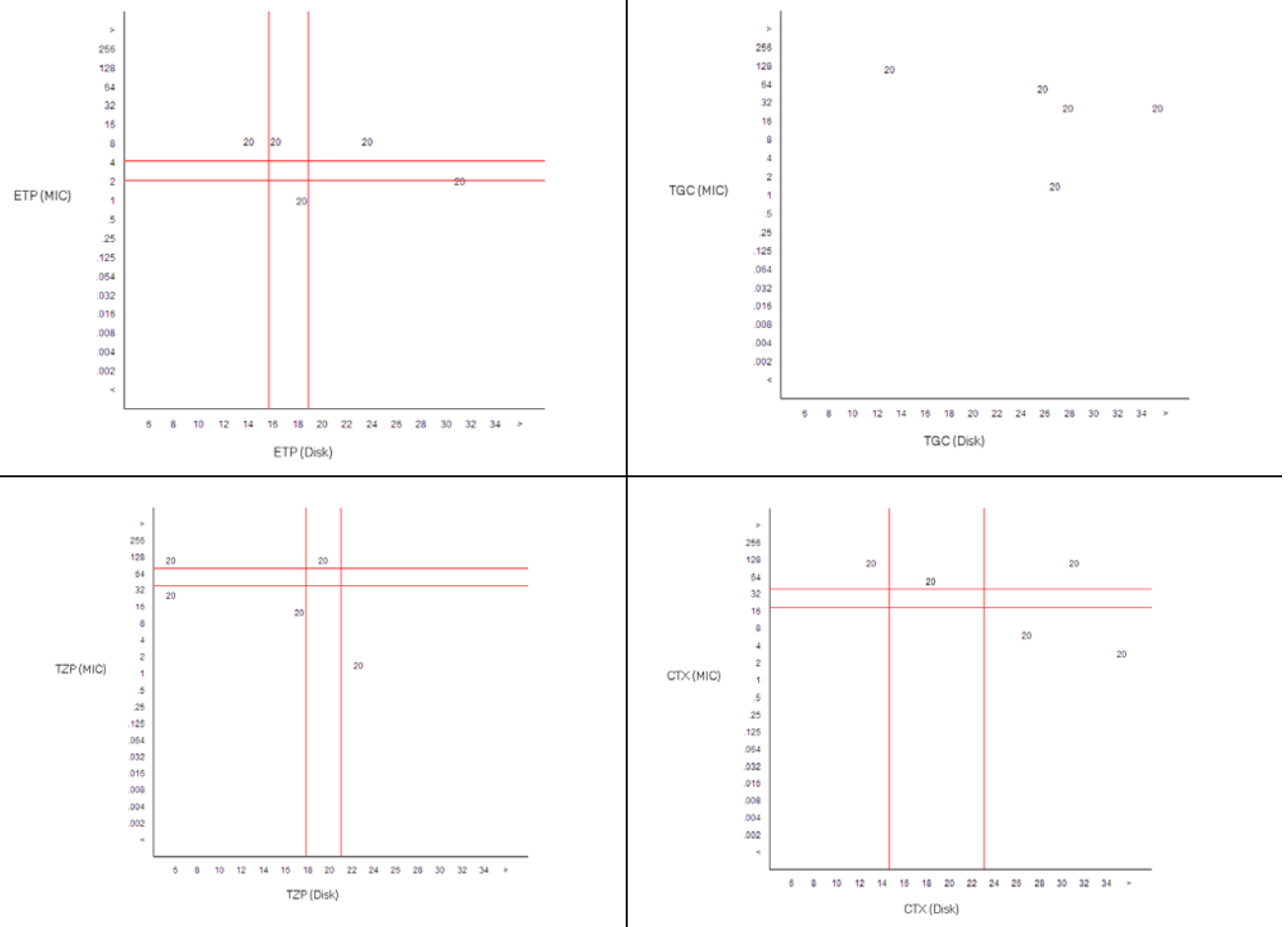


Figure 1: Concordance curves of *Bacteroides* sp. strains for the tested antibiotics (the numbers occurred inside the dotplots are representing the percentage of the bacterial strains out of the total number of strains, exhibiting a certain MIC and respectively, values of growth inhibition area. The vertical red lines are limiting the breakpoints values for inhibition zone diameters (mm), while the horizontal ones are corresponding to the MIC breakpoints values (µg/ml).

But a correct therapeutic approach requires that as soon as the etiological infectious agents are isolated, the susceptibility patterns of the tested isolates and the empirical therapy readjusted, if necessary, in concordance with the laboratory findings (de-escalation method). Till to-day, the testing of anaerobic strains susceptibility to antibiotics still remains very difficult for different reasons [21]: i) the anaerobic bacteria are slow growing, fastidious microorganisms; (ii) the anaerobic organisms are often part of a mixed infection and it may take several days to isolate and purify anaerobic colonies in order to embark on testing; iii) many laboratories do not perform enough testing of anaerobes to be able to maintain expertise. (iv) the methods established by the

NCCLS/CLSI have changed over the past decade, and the use of agar dilution by comparison with broth dilution has been somewhat controversial.

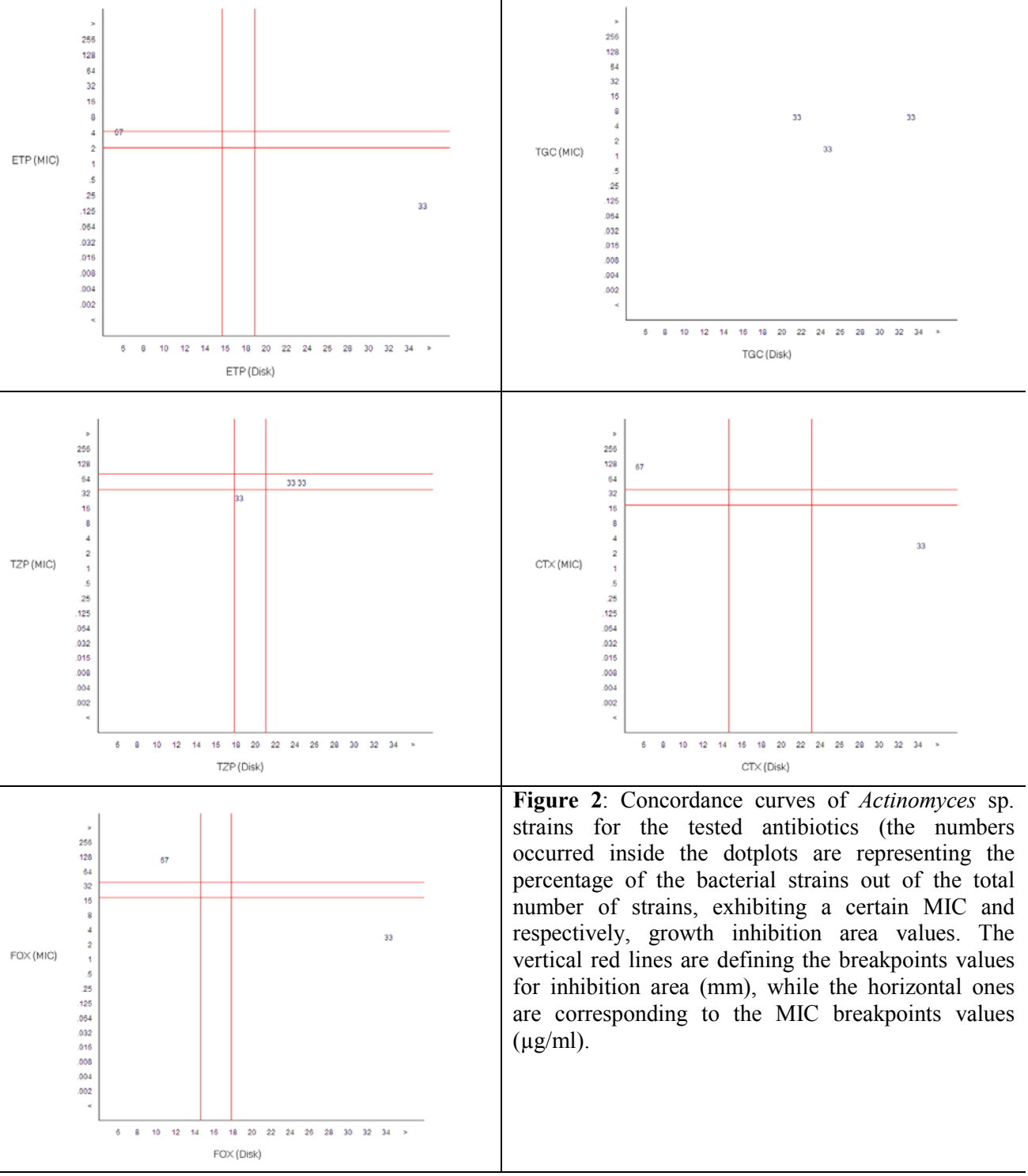


Figure 2: Concordance curves of *Actinomyces* sp. strains for the tested antibiotics (the numbers occurred inside the dotplots are representing the percentage of the bacterial strains out of the total number of strains, exhibiting a certain MIC and respectively, growth inhibition area values. The vertical red lines are defining the breakpoints values for inhibition area (mm), while the horizontal ones are corresponding to the MIC breakpoints values (µg/ml).

The development of the epsilometer (Etest) testing methodology has been helpful for the individual clinical laboratories in order to perform susceptibility testing of individual or selected clinical isolates to a single agent; nevertheless, the most hospital laboratories do not perform such testing. Therefore, the use of a simple, rapid and reliable method for the routine testing or in order to survey the antibiotic susceptibility of the anaerobic strains to a panel of antimicrobial agents may be very helpful for the routine practice of the clinical laboratories.

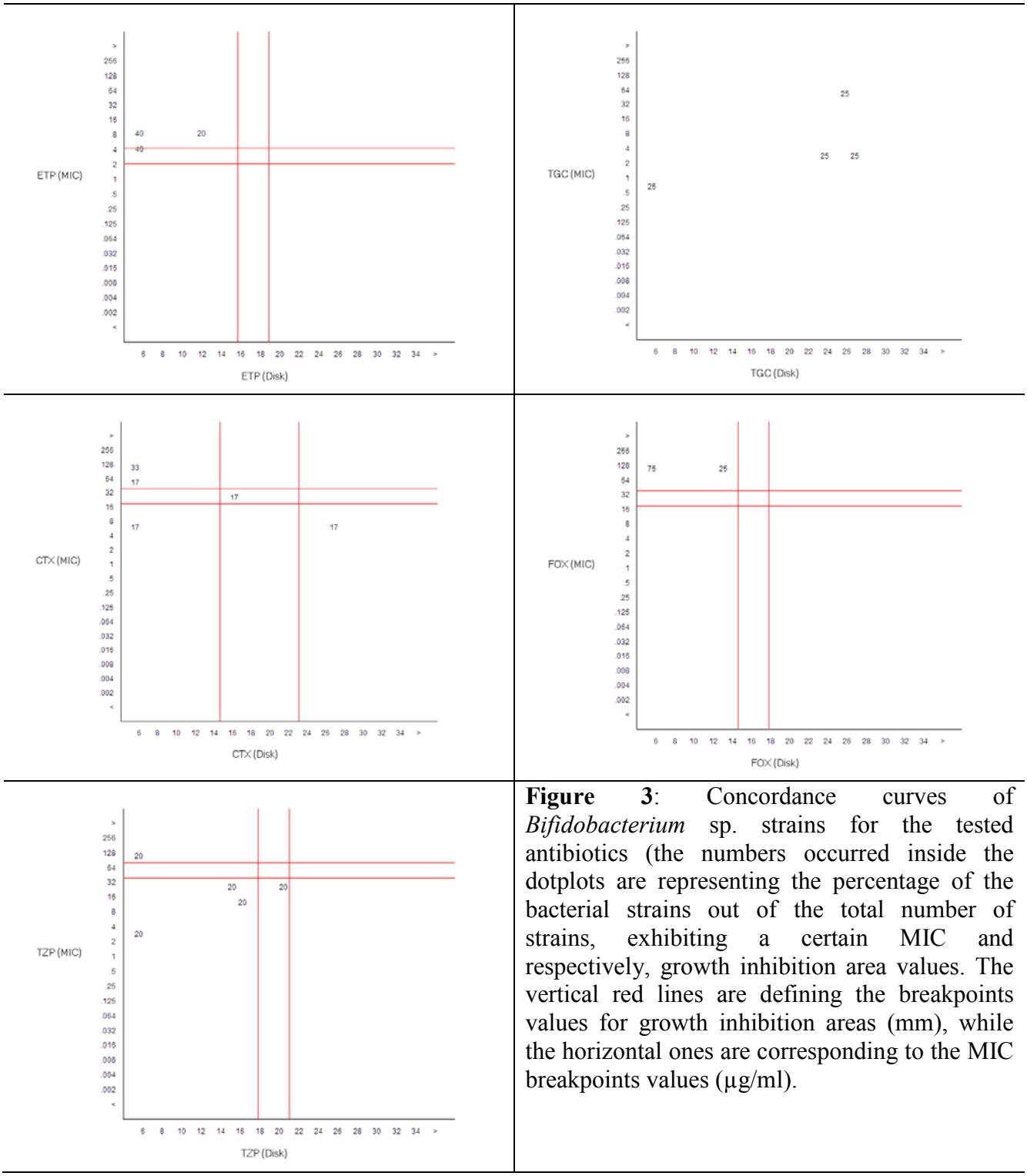


Figure 3: Concordance curves of *Bifidobacterium* sp. strains for the tested antibiotics (the numbers occurred inside the dotplots are representing the percentage of the bacterial strains out of the total number of strains, exhibiting a certain MIC and respectively, growth inhibition area values. The vertical red lines are defining the breakpoints values for growth inhibition areas (mm), while the horizontal ones are corresponding to the MIC breakpoints values (µg/ml).

We present here the results of antibiotic susceptibility testing in anaerobic strains, obtained by comparison with the broth microdilution versus a simple, disk diffusion method. The obtained results have been analyzed and correlated using the Data analysis panel provided by Whonet 5.5. The scatter plot analysis could establish the breakpoints for the tested strains in case of FOX, CTX, ETM, TZP, but not for TGC (figures 1, 2, 3). The tested *Bacteroides* sp. strains exhibited high resistance rates to ETM and TZP (20% of strains susceptible) (figure 1), *Actinomyces* sp. strains to TZP (no susceptible strain) (figure 2), whereas the *Bifidobacterium* sp. strains exhibited high resistance rates to FOX, TZP and ETM (no susceptible strain) (figure 3). However, the percentage of the susceptible

strains for the tested antibiotics reached in the best scenario 40%, in case of *Bacteroides* strains, for FOX and CTX, indicating the emergence of resistant strains to the majority of antibiotics used in the pre-operatory, empiric therapy. Although for TGC the breakpoints for S, I and R categories could not be established, the positioning of the highest number of the tested strains in the middle or the down right side of the scatter plots could indicate the highest susceptibility levels of the tested strains, as compared to the other four tested, especially in case of *Bifidobacterium* and *Actinomyces* strains.

The average MIC values of the tested strains were in all cases higher than the MIC S breakpoints value (CLSI), also supporting the emergence of resistant strains among the bacterial populations circulating in the analyzed area (figure 4).

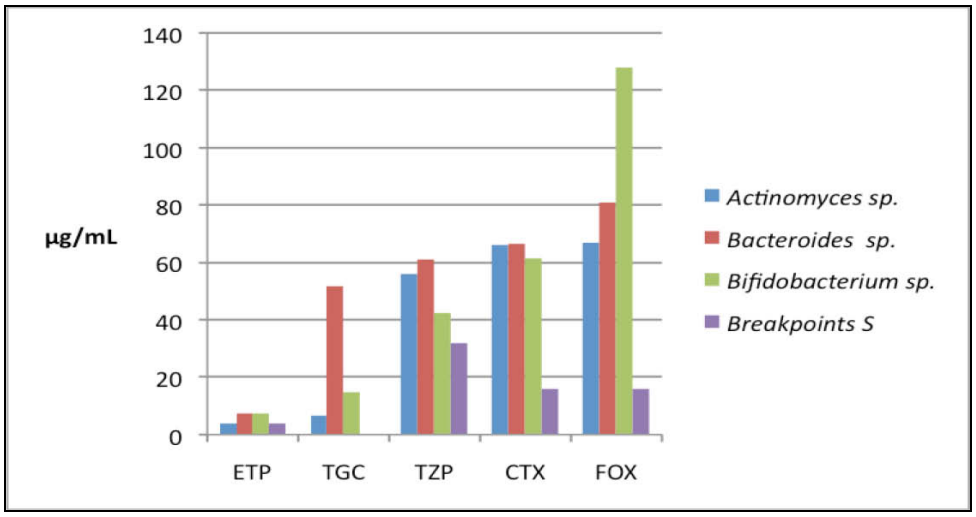


Figure 4: Graphic representation of the average MIC values of anaerobic strains, by comparison with the MIC breakpoints S value, as indicated by CLSI.

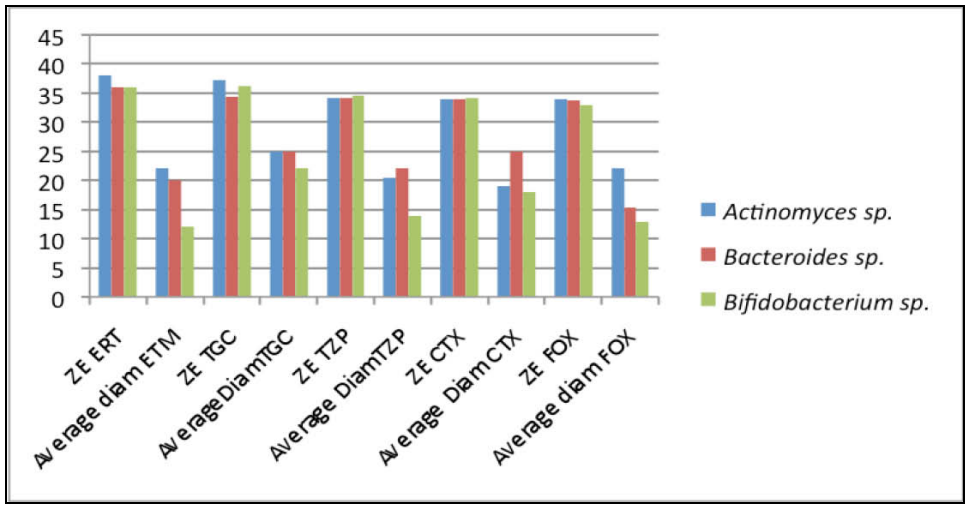
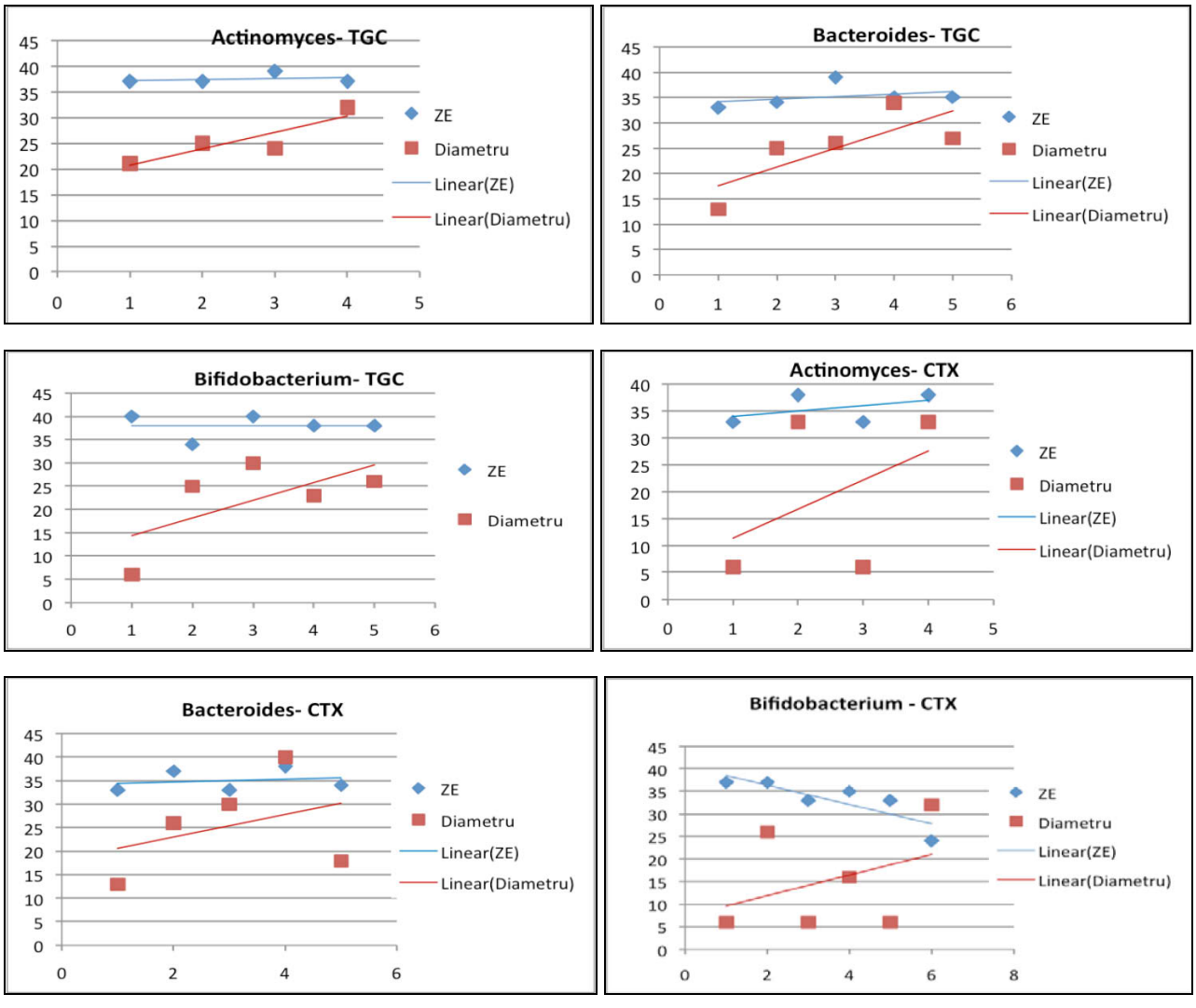


Figure 5: Graphic representation of the average values of the growth inhibition areas (mm) of anaerobic strains, by comparison with their calculated epidemiological cut-off values (ZE).

These results are also proved by the average values of growth inhibition areas, always inferior to the calculated ZE value (figure 5). It must be mentioned the good correlation achieved between the results obtained for all five antibiotics, when standard CLSI method of liquid medium microdillutions and the disk diffusion method, adapted to the anaerobic conditions (Bailey and Scott 2002). These results are demonstrating the possible use of the disk diffusion method in the routine practice for testing the antibiotic susceptibility of the anaerobic strains isolated from the emergency high risk severe cases. In this way the efficiency of the pre-operatory antibiotic therapy control (recommended by international guidelines) could be increased and readjusted (de-escalation) to the

local susceptibility profiles of the isolated bacterial strains. The comparative analysis of the linear trendline of zone inhibition diameters and respectively calculated ZE (figure 6) value for the microbial strains belonging to different species against the tested antibiotics, showed that: i) as much as closer to the ZE value the trendline of the inhibition zone (determined by a certain antibiotic upon a circulating bacterial strain) is, the more efficient the respective antibiotic must be considered; ii) the susceptibility levels to a certain antibiotic among the strains belonging to the same species are different. The greatest efficiency was noted for TGC on all three species of strains and for ETM only on *Bacteroides* spp. and *Actinomyces* spp. strains. Less efficient proved to be the cephalosporins of the second (FOX) and third generation (CTX) upon the Gram-positive bacterial strains. Concerning TZP, our results indicated that the great majority of the studied strains belonging to all three species proved to be resistant to this antibiotic, when the susceptibility was tested by the both methods although the recent literature data have recommended this antibiotic as very active on all isolated anaerobic bacteria. These results require as necessary the establishment of the antibiotic susceptibility profiles of the bacterial populations circulating in a certain geographical area, in order to assure the proper selection of the empiric antibiotherapy, especially in severe infections. Taken together, the analysis of the MIC values, zone inhibition diameters and the calculated epidemiological cut-off values (ZE) of anaerobic strains isolated from intra-abdominal severe infections, belonging to *Bacteroides, Actinomyces* and *Bifidobacterium* species indicated as the most efficient antibiotics TGC against all anaerobic strains, irrespective to their taxonomic affiliation, ETM against *Actinomyces* and CTX against *Bacteroides*. The less efficient antibiotics proved to be the second generation cephalosporins (cefoxitin) as well as the piperacillin + tazobactam association.



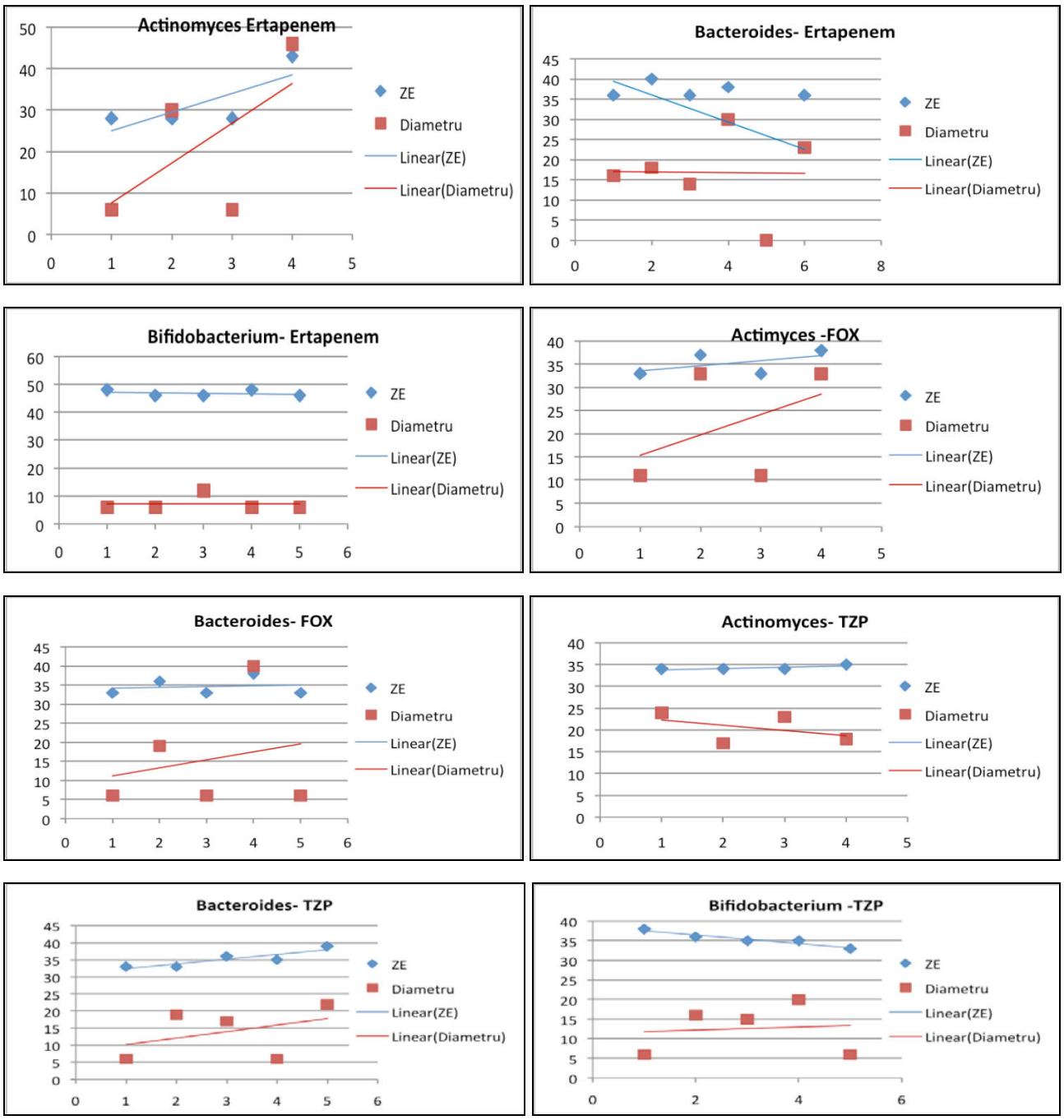


Figure 6: Graphic comparative representation of linear trendlines of zone inhibition diameters and calculated ZE for the anaerobic bacterial strains.

4. Conclusions

The corner stones in the management of 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. The analysis of the MIC values, the growth inhibition areas and the calculated epidemiological cut-off values (ZE) of the anaerobic strains belonging to *Bacteroides* sp., *Bifidobacterium* sp. and *Actinomyces* sp. strains isolated from intra-abdominal severe infections, indicated tigecycline as the most efficient atntibiotic on all three species, followed by ertapenem and the third generation cephaloaporins, while the less efficient were the second generation cephalosporins, as well as piperacillin+ tazobactam association. The present results are

recommending for the current practice the disk diffusion method as the most simple and reliable method for testing the antibiotic susceptibility of anaerobic bacteria isolated from severe infections in order to survey the emergence of new resistance aspects in a certain limited area and to readjust, if necessary, the set of antibiotics to the new epidemiological conditions. A good correlation between the results of antibiotic susceptibility testing obtained by the standard microdillution method recommended by CLSI and the disk diffusion method adapted to anaerobic conditions was obtained, both methods indicating tigecycline as the most active antimicrobial agents on all three studied anaerobic species. The permanent surveillance of the efficiency of the antibiotics used in the pre- and post-operatory treatment can be achieved only by close collaboration between clinicians and clinical microbiology laboratory.

5. References

[1] Sartelli M., Viale P., Koike K., Pea F., Tumietto F., van Goor H., Guercioni G., Nespoli A., Tranà C., Catena F., Ansaloni L., Leppaniemi A., Biffl W., Moore F.A., Poggetti R., Pinna A.D., Moore E., WSES consensus conference: Guidelines for first-line management of intra-abdominal infections, *World Journal of Emergency Surgery*, 6, 2, **2011.**

[2] Crociani, F., Biavati B., Allessandrini A., Chiarini C., and Scardovi V., *Bifidobacterium inopinatum* sp. nov. and *Bifidobacterium denticolens* sp. nov., two new species isolated from human dental caries, *Int. J. Syst. Bacteriol.*, 46, 564-571, **1996.**

[3] Bailey and Scott: Diagnostic Microbiology Mosby-Elsevier eleventh Edition, 511- 536, **2002.**

[4] Brook I., Frazier EH. Significant Recovery of nonsporulating Anaerobic Rods from Clinical Specimens. *Clin.Infect. Diseases*, 16, 4, **1993**.

[5] Verma R., Dhamija R., Ross S.C., Batts D.H., Mark E., Loehrke S. Symbiotic bacteria Induced necrotizing Pancreatitis. *J. Pancr.*,11, 5, 474-476, **2010.**

[6] Green S.L. Casereport: fatal anaerobic pulmonary ibfection due to *Bifidobacterium eriksonii*, *Postgrad.Med.,* 63, 187-188, **1978**.

[7] Nakazawa T., Kaneko K., Takahashi H., Inoue S. Neonatal meningitis caused by *Bifidobacterium breve*. Brain and Development, 18, 2, 160-162, **1996.**

[8] Gyoung Yim Ha, Chang Heon Yabg, Heesoo Kim, Yunsop Chong: Case of sepsis caused by *Bifidobacterium langum*. *J. Clin. Microbiol*., 37, 4, 1227-1228, **1999.**

[9] Cryan B., Azadian B.S., Dunwoody G.W., Richards P.G. Epidural abcess due to *Streptococcus milleri* and *Bifidobacterium* species, *J.Infect.*, 22, 214-215, **1991**.

[10] Dorsher C. W., J. E. Rosenblatt W. R. Wilson, Ilstrup D. M., Anaerobic bacteremia: decreasing rate over a 15-year period, *Rev. Infect. Dis.*, 13, 633-636, **1991**.

[11] Brook I. Indigenous microbial flora of humans. In: Surgical Infectious Diseases. 3rd ed. Norwalk, Conn: Appleton & Lange, 37, **1995.**

[12] Wexler H.M., Finegold S.M. Current susceptibility patterns of anaerobic bacteria, *Yonsei Med J.,* 39, 6, 495-501, **1998**.

[13] Jousime-Somers H., Summanen P., Citron D.M., Belmont, Calif. Wadsworth-KTL Anaerobic Bacteriology Manual. 6th ed. Star Publishing; **2002**.

[14] Brook I. Enhancement of growth of aerobic and facultative bacteria in mixed infections with *Bacteroides* species, *Infect Immun*., 50, 3, 929-31, **1985**.

[15] Durmaz B., Dalgalar M., Durmaz R. Prevalence of enterotoxigenic *Bacteroides fragilis* in patients with diarrhea: a controlled study. *Anaerobe*, 11, 6, 318-21, **2005.**

[16] Çoban, Yetkin G., Kebudi A. Abdominal Actinomycosis: a Case Report, *Acta Chir Belg*., 102, 521-523, **2002.**

[17] Yeguez J. F., Martinez S. A., Sands L. R., Hellinger M. D. Pelvic actinomycosis presenting as malignant large bowel obstruction : a case report and a review of the literature. *Am Surg*.,, 66, 85- 90, **2000.**

[18] Bailey and Scott's Diagnostic Microbiology eleventh Ed., Mosby Inc., **2002**.

[19] CLSI: Methods for Antimicrobial Susceptibility Testing of Anaerobic bacteria, Approved Standard Seventh Edition. Vol. 27, no. 2.
[20] Goldstein E.J.C., Snydman D.R., Intra-abdominal infections: review of the bacteriology, antimicrobial susceptibility and the role of ertapenem in their therapy. *Journal of Antimicrobial Chemotherapy.,*53, Suppl. S2, **2004.**
[21] Rosenblat, J. E., Brook, I., Clinical relevance of susceptibility testing of anaerobic bacteria, *Clinical Infectious Diseases.,* 16, 4, 446–8, **1993.**