British Journal of Medicine & Medical Research
15(4): 1-14, 2016, Article no.BJMMR.25281
ISSN: 2231-0614, NLM ID: 101570965

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Microbiota Evaluation and Extracellular Cytokine Profile in Patients Affected with Intraabdominal Infection

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SGS and JCS designed the study, wrote the protocol, and wrote the first draft of the manuscript; authors JBRN and RAPS evaluated and collected the patient's specimens; authors JFGF, JGS and HS managed the literature searches and performed the microbiological and immunological analysis. Authors SGS and MARC managed all steps of experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/25281
Editor(s):
(1) Alexandre Zanchenko Fonseca, Department of General Surgery, Univertisty of Santo Amaro, Brazil.
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Reviewers:
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(3) S. Thenmozhi, Periyar University, India.
Complete Peer review History: http://sciencedomain.org/review-history/14309

Original Research Article

Received 26th February 2016
Accepted 16th April 2016
Published 25th April 2016

ABSTRACT

Failures in the control of infectious focus may be associated with Intra-abdominal infections (IAI)-driven sepsis. We evaluated the bacterial antimicrobial profile and the cytokine production in patients with IAI in Belo Horizonte, Brazil. To the analyses, Vitek 2 bioMérieux and BD-CBA

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Human Inflammatory Cytokines were used. *Escherichia coli*, *Enterococcus faecalis* and *Bacteroides fragilis* were predominant in this cohort. Enterobacteriaceae was resistant to at least 4 different antimicrobial classes and 80.0% of *Acintobacter baumannii* strains to imipenem. 81.8% of *Staphylococcus* spp. were methicillin-resistant. Penicillin and clindamycin resistance were found in 80.0% and 26.7% of anaerobes, respectively. IL-8 was found in all IAI secretions and in 93.5% of analyzed sera; while IL-6 was identified in 93.5% of patient's serum and in 51.6% analyzed secretions. IL-10 was detected in 53.3% of patient's serum. Our data indicates the relevance of further cytokine profile studies to better understanding the evolution of these processes.

Keywords: Intraabdominal infection; microbiogical study; citokines profile; anaerobes.

1. INTRODUCTION

Intra-abdominal infections (IAI) are still regarded as an important cause of morbidity and mortality worldwide. This is the second cause of severe sepsis in Intensive Care Units [1,2,3,4]. Microbial etiology of IAI depends on the intestine breaking point, intraabdominal organs affected, patient's immune status, possible changes the microbiota conditioned by prior administration of antibiotics, and associated comorbidities [5,6]. These infections are caused mostly by Gram negative, aerobic and anaerobic mandatory, and often polymicrobial [7]. Although common species of IAI depends on the anatomic site of infection [5], it is usually associated with intestinal microbiota species like *Escherichia coli*, *Klebsiella* spp., *Enterococcus* spp., among others [4,5,6]. Importantly, hospitalized patients are often infected by antimicrobial-resistant microorganisms from the health unit's own microbiota, which may include *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter* spp., *Enterobacter* spp., *Staphylococcus aureus* resistant to methicillin (MRSA), *Enterococcus* spp., *Candida* spp., and *Klebsiella* producing β-lactamases of broad-spectrum (ESBL) [5,8].

Early diagnosis of the IAI is important to assess severity and improve prognosis of disease. Factors influencing progression of IAI include advanced age, malnutrition, pre-existing diseases, use of immunosuppressive, prolonged peritonitis, virulence of the offending microorganism, among others. Factors that prolong hospitalization and susceptibility to nosocomial infections are also associated with bad evolution [1,9,10].

Antimicrobials are usually prescribed immediately after IAI is suspected, even before the diagnosis is confirmed [11,12]. Thus, antibiotic therapy to treat IAI always involves a delicate balance between optimization of empirical therapy to improve clinical outcomes and a reduction in the excessive use of antimicrobial agents, which can lead to selection of resistant antimicrobial strains [8,11,12]. Antimicrobial resistance has been identified as a key challenge in treatment of IAI. Production of β-lactamases by various microorganisms, including pathogenic anaerobic, increased appreciably in the last 20 years. For example, most strains of *B. fragilis* are resistant to cephalosporins because their β-lactamases are cephalosporinases [9,13].

The high mortality from severe sepsis and septic shock is closely related to inappropriate approach to IAI. Poorly controlled infection can cause excessive inflammation, which is the mechanism of septic shock. The focus of therapy should be to allow host defenses control infection keeping excessive inflammation on check, which can be achieved by appropriate antibiotic therapy [1,2].

Because of the clinical relevance of IAI, this study evaluated the microbial composition of these processes, including the investigation of anaerobic bacteria, and its susceptibility profile to antimicrobials, as well as the inflammatory profile of patients with IAI.

2. MATERIALS AND METHODS

2.1 Clinical Specimens

Clinical specimens were obtained from 51 patients with different IAI attended at the Hospital of Military Police of Minas Gerais state (HPM), Risoleta Tolentino Neves, Clinical Hospital of the Federal University of Minas Gerais (HRTN/UFMG; HC/UFMG) and the Center of Specialization in Ultrasonography (CEU), from March 2011 to October 2012, in Belo Horizonte city, Minas Gerais state, Brazil.

Intra-abdominal secretions were obtained by puncture with needle and syringe from the

affected site during surgery, or aspiration guided by ultrasound at the drainage procedure. Samples was divided in two aliquots, one in Ringer Pre-reduced Sterilized Anaerobically medium for anaerobic culture [14], and other aliquot in sterile tubes for immunological analysis and aerobic culture. It was also obtained 20 ml of blood from each patient, using vacuum sterile disposable tubes (Vacutainer). Sera were obtained by centrifugation of the blood samples at 800 g for 10 min at 4℃ and stored at -20℃ until use. These clinical materials were immediately transported to the Laboratory of Microbiology and Oral Anaerobic in ICB/UFMG.

2.1.1 Inclusion criteria

Patients experiencing diverse intraabdominal infections diagnosed by the responsible physician and have signed the Term of Informed Consent. Only biological samples collected and transported to the laboratory in anaerobic conditions were processed for anaerobic culture.

2.1.2 Exclusion criteria

Patients who do not presented intraabdominal infections diagnosed by the responsible physician and those who do not agreed to participate of this study. Biological samples that were not collected and transported to the laboratory in anaerobic conditions were not included in this study.

2.2 Ethical Considerations

This study was approved by the Ethics Committee of the Federal University of Minas Gerais (Report no; ETIC 0097.0.203.000-10). Informed consent was obtained for every adult and informed assent plus informed consent of legal guardians were obtained for every child.

2.3 Microorganisms Isolation

Culture of anaerobic bacteria was performed in the anaerobic chamber with atmosphere of 5% CO_2, 10% H_2 and 85% N_2 (Forma Scientific Anaerobic System # 1025). The following selective mediums were used: Brucella Agar Medium supplemented with 5 mg/ml hemin, 1mg/ml menadione and 5% sheep blood-BRU-S; Bacteroides Bile Agar-Esculin supplemented with 5 mg/ml hemin, 1 mg/ml and 2.5 ml menadione/L Gentamicin-BBE; Phenylethanol Agar supplemented with 5 mg/ml of hemin, 1 mg/ml menadione-PEA and 5% sheep blood agar and Omata agar, composed of 1.5% trypticase peptone, 0.5% yeast extract, 0.5% glucose, 0.5% sodium chloride 0.075% L-cystine 0.001% crystal violet, 0.001% streptomycin sulfate, 1.5% agar and 5% horse serum [14].

The culture of aerobic facultative bacteria were processed in a laminar flow hood (Veco microbiological Biosafe I) using rich medium Trypticase Soy Agar supplemented with 5% horse blood; MacConkey and Mannitol selective mediums [14,15].

2.4 Bacterial Identification and Antimicrobial Susceptibility Testing

The determination of antimicrobial susceptibility profile of aerobic bacteria was performed by the automated system Vitek II using specific cards CGP (AST-P612) and BGN (AST-N105).

For anaerobic bacteria analyses, the minimum inhibitory concentration (MIC) of drugs able to inhibit microbial growth was determined by the agar dilution method [16] (CLSI, 2015). The *B. fragilis* ATCC 25285 and *Eubacterium lentum* ATCC 43055 reference strains were used as experiment controls. The antibiotics tested were penicillin (PEN), piperacillin/tazobactam (PTZ), imipenem (IMI), cefoxitin (CFO), clindamycin (CLI) and metronidazole (MET). The interpretation of results was performed according to CLSI criteria in 2015 [16].

2.5 Immunological Analysis

2.5.1 Dosage of cytokine using Fluorescence Activated Cell Sorter (FACS)

Cytokines IL-1, IL-6, IL-8, IL-10 and TNF in the serum and abdominal secretions were assessed with BD™CBA Human Inflammatory Cytokines kit, according to manufacturer's instructions. The levels of cytokine production were examined and compared to several clinical/laboratory parameters in order to determine possible relationships between clinical patients with IAI and inflammatory cytokine production.

2.6 Statistical Analysis

Statistical analyses were performed with the GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California). The calculations used were the Mann-Whitney test and Kruskal-Wallis test. The level of significance was $p < 0.05$.

3. RESULTS

3.1 Clinical Dates

Patients with IAI were recruited from hospitals and clinics of Belo Horizonte. From 51 patients, 27 were men, mean age was 42.3 years old (ranging from 14 to 88 years). Of note, age was missing from 5 patients. More than half (29/51) reported some underlying disease or previous recent surgery, being bariatric surgery the most common procedure in this cohort. The common co-morbidities were hypertension (n=5) and diabetes (n=4). Antimicrobial use information was available for 38 of the 51 patients. Of the 38 patients, 89.5% (34/38) were under antibiotics by the time of sample collection being metronidazole the most used, followed by gentamicin and ceftriaxone. There was predominance of abdominal abscesses, (19/51) peritoneal collections (13/51), appendicitis (8/51), peritonitis (4/51) and others (7/51) (Table 1).

A subset of 21 patients from only one of hospitals was further analyzed. We found that 7/21 (33.3%) had nosocomial infection while 14/21 (66.7%) had community-acquired infection. Individuals with nosocomial infections displayed increased length of hospitalization (31 ±16 days) when compared to community-acquired infections (8 ±8 days) ($p < 0.0007$).

3.2 Prevalence of Microorganisms

3.2.1 Microbiological culture of intra-abdominal secretions

Microbial growth was observed in 64.7% (33/51) of the analyzed specimens, despite the prior use of antibiotics for at least 63.6% (21/33) of the positive samples. We recovered 88 microorganisms, confirming the polymicrobial nature of these infections. The average recovery was 2.7 species or strains per positive sample.

Table 1. Demographic and clinical variables of the patients with intraabdominal infection (n=51)

Age, years (X±SD)	42,3±20,4
Men, n (%)	27 (52.9)
Major comorbidities and recent surgeries, n (%)	
Abdominal surgeries	13 (25.5)
Systemic arterial hypertension	5 (9.8)
Diabetes mellitus Type 2	4 (7.8)
Clinical specimens, n (%)	
Abdominal abscesses	19 (37.3)
Peritoneal collections	13 (25.5)
Appendicitis	8 (15.7)
Peritonitis	4 (7.8)
Others	7 (13.7)
Antimicrobials used by 38 patients with information of use, n (%)	
Metronidazole	22 (57.9)
Gentamicin	8 (21.1)
Ceftriaxone	8 (21.1)
Vancomycin	5 (13.2)
Meropenem	4 (10.5)
Cefepime	4 (10.5)
Ciprofloxacin	4 (10.5)
Fluconazole	2 (5.3)
Cephalothin	2 (5.3)
Clindamycin	1 (2.6)
Ampicillin	1 (2.6)
Cefazolin	1 (2.6)
Ertapenem	1 (2.6)
Polymyxin B	1 (2.6)
Linezolid	1 (2.6)
Tigecycline	1 (2.6)

Monomicrobial cultures were only 27.3% (9/33) of the cases. We were able to identify 83 of the 88 microorganisms recovered, which were divided into 20 genera and 35 species. According to the phenotypic identification, obtained by Vitek System II, anaerobic bacteria were present in 39.4% (13/33) of the positive cultures, aerobic bacteria in 90.9% (30/33) of the cases represented by 64 bacteria and yeasts in 9.1% (3/33) of cultures (Table 2).

Among the anaerobes *Bacteroides fragilis* group (n=8) predominated, followed by *Prevotella* spp. (n=5) and *Fusobacterium nucleatum* (n=2). Among the aerobic, *E. coli* (n=6), *Enterococcus faecalis* (n=6), *Acinetobacter baumannii* (n=5), *Sphingomonas paucimobilis* (n=5) and *S. epidermidis* (n=5) were the most frequent. The three yeasts recovered were *Candida* spp.

Table 2. Microorganisms recovered from the 33 positive cultures of 51 patients with clinical intraabdominal infection

Microorganisms	N°of samples recovered	%
Escherichia coli	6	6.81
Enterococcus faecalis	6	6.81
Bacteroides fragilis	5	5.68
Sphingomonas paucimobilis	5	5.68
Acinetobacter baumannii	5	5.68
Staphylococcus epidermidis	5	5.68
Prevotella bivia	3	3.41
Streptococcus anginosus	3	3.41
Proteus mirabilis	3	3.41
Gemella morbilorum	3	3.41
Pediococcus pentosaceus	3	3.41
Streptococcus pluranimalium	2	2.27
Kocuria kristinae	2	2.27
Streptococcus constellatus	2	2.27
Staphylococcus aureus	2	2.27
Providencia stuartii	2	2.27
Citrobacter freundii	2	2.27
Fusobacterium nucleatum	2	2.27
Staphylococcus warneri	2	2.27
Candida albicans	2	2.27
Staphylococcus haemolyticus	2	2.27
Enterococcus faecium	2	2.27
Candida kefyr	1	1.13
Bacteroides ovatus	1	1.13
Bacteroides vulgatus	1	1.13
Bacteroides thetaiotaomicron	1	1.13
Streptococcus sanguinis	1	1.13
Streptococcus dysgalactiae	1	1.13
Hafnia alvei	1	1.13
Morganella morganii	1	1.13
Prevotella melaninogenica	1	1.13
Prevotella intermedia	1	1.13
Propionibacterium acnes	1	1.13
Providencia rettgeri	1	1.13
Pseudomonas aeruginosa	1	1.13
Pseudomonas spp	1	1.13
GPC NI	3	3.41
GNR NI	2	2.27
Total	88	100

Legend: GPC: Gram Positive Cocci; GNR: Gram Negative Rods; NI: Not Identified

Associations between aerobic and anaerobic were observed in nine (69.2%) of 13 patients with positive cultures for anaerobes, and the association between anaerobic bacteria was observed only in 3 of the 13 positive cultures. In addition, 10 cases were of polymicrobial infections.

3.3 Susceptibility Profile of Antimicrobial Agents

3.3.1 Aerobic Gram Positive Cocos (GPC)

We observed that from 11 *Staphylococcus* spp. strains, nine were resistant to oxacillin, two of them were *S. aureus* and seven were coagulase negative *Staphylococcus* spp. regarding to *Enterococcus* strains, only one was resistant to ampicillin, but in contrast, all were resistant to clindamycin and trimethoprim/sulfamethoxazole. Vancomycin resistance was observed in one strain of *E. faecium*, which was also resistant to most of the other drugs being sensitive to only tetracycline and gentamicin synergy testing from the 13 antimicrobial agents tested. Daptomycin was the most effective antimicrobial against *E. faecalis* strains.

3.3.2 Aerobic Gram Negative Rods (GNR)

A total of 25 GNR strains were analyzed as to their antimicrobial susceptibility profile. None of the 17 antimicrobial agents tested, represented for five classes and four subclasses, showed to be effective against all strains tested. High rates of resistance to 2nd and 3rd generation cephalosporin were observed between the GNR strains, exceeding 50% for cefotaxime. Even for cefepime, a 4th generation cephalosporin, the rate was high, approaching 40%.

Of the Enterobacteriaceae (n=16), the highest resistance levels were observed to cephalothin (n=13), ampicillin (n=12), ampicillin+sulbactam (n=11) and colistin (n=9). Lower rates of resistance were obtained for the carbapenems and piperacillin+tazobactam. Three out of six *E. coli* strains was ESBL producers. Among the aerobic non-fermenting GNR strains, the resistance rates (full or intermediate) were observed to a larger number of drugs. Seven of the nine tested microorganisms were multiresistant, being one *A. baumannii* and one of *P. aeruginosa* strains, sensitive to only one antimicrobial each, ampicillin+sulbactam and colistin, respectively. The colistin, tigecycline and meropenem were the antimicrobials that performed better against non-fermenters BGR.

3.3.3 Anaerobic bacteria

All *Bacteroides* spp. strains (n=8) were resistant to penicillin, seven of these were ESBL positive bacteria. *B. ovatus* strain showed the greatest resistance level, 3/6 antibiotic tested. The two samples of *Fusobacterium nucleatum*, one of them ESBL producer and one of *Prevotella* spp., were sensitive to the six antimicrobial agents tested. Four samples of *Prevotella* spp. were only resistant to penicillin. One anaerobic BGP recovered, *Propionibacterium acnes*, was found to be resistant only to metronidazole (Table 3).

3.4 Cytokines Analysis

In order to gain insight about the inflammatory processes associated with these infections, we evaluated a panel of proinflammatory cytokines in intraabdominal secretions (n=30) and sera (n=31) of these patients by multiplex inflammatory CBA kit (BD). As expected, the levels of proinflammatory cytokines in the abdominal secretion were higher when compared to serum levels (Table 4). For example, the median concentration of IL8 was 392,255.89 pg/mL in abdominal secretion against only 82.98 pg/mL in serum (P<0.001). For IL1, abdominal secretion displayed a median concentration of 20,122.23 pg/mL while serum concentration was 0.99 pg/mL (P<0.001). Actually, IL1 was rarely detected in serum (6/31 samples) at levels above theoretical limit of detection of the kit. On the other hand, IL10 was more likely to be detectable in serum than in secretion. For example, only 7% (2/30) of the patients showed IL10 levels above detection limit in abdominal secretion, while 52% (16/31) patients had IL10 detected in the serum. In addition, the geometric means of IL10 levels was 14 times higher in serum (2.59 pg/mL, CI: 0.91-11.59) than in secretion (0.18 pg/mL, CI: 0.06-0.71).

The isolation of microorganisms from the culture had a big impact on the cytokine profile in abdominal secretion. When compared to negative culture samples, positive samples displayed about 5 times more IL8 (GM 675.314,59 vs 13.2341,53, P=0.0024) and around 91 times more IL1b (GM 105.626,82 vs 1.157,39; P<0.0003) in abdominal secretion (Fig. 1). In addition, nosocomial-acquired infections were associated with higher pro-inflammatory profile showing higher TNF (p=0.03) and trend of higher IL-8 (p= 0.08) and IL-6 (p=0.09) than community-acquired infection (Fig. 2). Interestingly, no major differences were found in the other cytokines analyzed in serum levels.

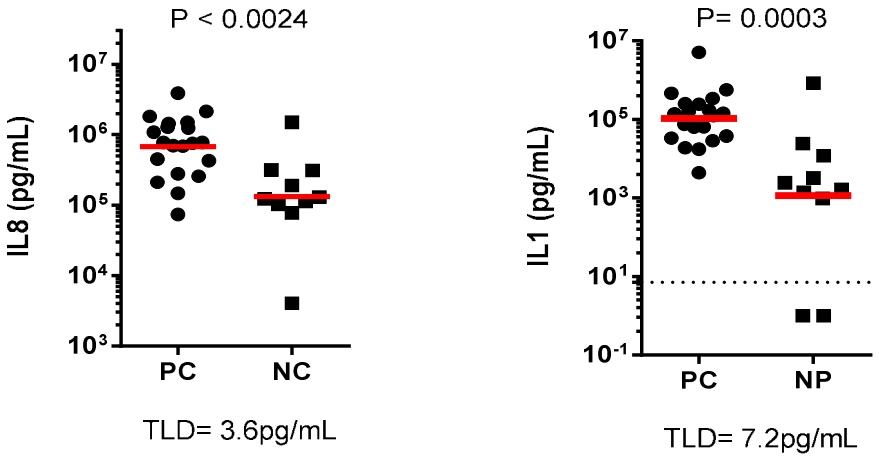

Fig. 1. Relationship between the positive culture and levels of cytokines IL-8 and IL-1 in the abdominal secretion of patients with intraabdominal infection

PC: Positive Culture; NP: Negative Culture; TLD: Theoretical Limit of Detection

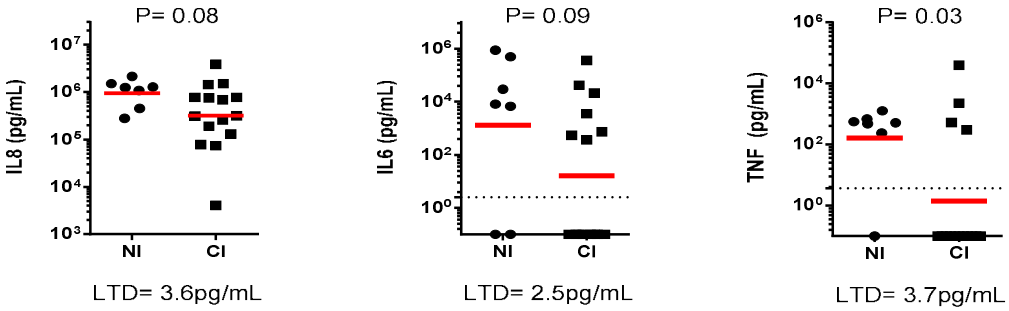

Fig. 2. Relationship between the origin of the intraabdominal infection and levels of cytokines IL-8, IL-6 and TNF

NI: Nosocomial Infection; CI: Community Infection

4. DISCUSSION

Studies have associated severe IAI with a significant mortality rate [1,2,3,17], which can be associated to several reasons, but inadequate empirical treatment and failures in the control of infectious focus leading to increased bacterial resistance may be the main factors responsible for this situation [12,18].

We analyzed 51 IAI secretion samples from patients treated in four healthcare centers in Belo Horizonte, MG, Brazil, from March 2011 to October 2012. From the specimens analyzed, 64.7% had positive cultures, averaging 2.7 microorganisms by clinical specimen. Despite increased knowledge in epidemiology, improved diagnostics and better antibiotics choice for treating these cases, the results were similar to those obtained 10 years ago by Santos et al. [19]. Sartelli et al. [20], in a multicenter study conducted in Europe between January and June 2012 with 2152 patients in 68 health facilities spread across all regions of Europe, also found similar results. These data show that IAI is still one of the major challenges to the health system.

In our study, *E. coli* was the most prevalent among GNR aerobic totaling 6.8%, and *E. faecalis*, among GPC aerobic, also representing 6.8%. Interestingly, despite high levels of aerobic bacteria in our samples, the presence of anaerobic bacteria is considered the major cause of abscess formation in IAI, with an important involvement of encapsulated *B. fragilis* [13,21,22].

In this study, we evaluated the susceptibility profile to antimicrobial GNR and GPC aerobic and anaerobic, 60 bacteria. The antimicrobial resistance rates among aerobic GNR varied from 5% (meropenem) to 91.3% (ampicillin), which were similar to those found by Santos et al. [22] in a previous study in the same region of Belo Horizonte. Cantón et al. [23], in a Spanish cohort from 2002 and 2010, also found meropenem to be the most effective antibiotic to aerobic BGN. For aerobic GNB, carbapenems were the most effective, being active even against strains of *E. coli* producing ESBL, confirming carbapenems as first choice empiric therapy of infections caused or strongly suspected to be caused by ESBL-producing Enterobacteriaceae [23].

Aerobic GNR non-fermenters, here represented by *A. baumannii*, *P. aeruginosa* and *S. paucimobilis*, showed high levels of antimicrobial resistance rates ranging from 11.1% (colistin) to 100% (ampicillin and cephalothin). In addition, half of them was resistant to 10 of the 13 antimicrobials tested. According to Bassetti et al. [24], multidrug-resistant bacterium is resistant to one or more antimicrobials from three or more different classes. It is known that infections involving GNR resistant to multiple drugs have few effective antimicrobial options and result in high mortality rates [23,24].

Coagulase positive *Staphylococcus* strains showed resistance rates ranging from 0% (nitrofurantoin and mupirocin) to more than 77% (erythromycin and oxacillin). In a mini-review by Gomes et al. [25], resistance rates of *S. epidermidis* ranged from 75-90% for methicillin.

Table 3. Susceptibility profile to antimicrobials (MIC 50 and 90%) of the anaerobic strain recovered from patients with clinical intra-abdominal infection

Microorganisms (n)	Antimicrobials	MIC (µg/ml)				Number (%)	
		Susceptibility range		Susceptibility		Resistance	
				50%	90%	Total	Intermediate
Bacteroides fragilis group	PPT	≤8	16	≤8	16	0	0
8	CFO	16	64	16	64	2(25)	1(12,5)
	IMI	2	4	2	4	0	0
	CLI	≤0,5	≥32	2	≥32	4(50)	0
	MET	8	8	8	8	0	0
	PEN	4	≥8	≥8	≥8	8(100)	0
Prevotella spp.	PPT	≤8	≤8	≤8	≤8	0	0
5	CFO	≤4	≤4	≤4	≤4	0	0
	IMI	≤1	2	≤1	2	0	0
	CLI	≤0,5	≤0,5	≤0,5	≤0,5	0	0
	MET	4	8	8	8	0	0
	PEN	1	≥8	≥8	≥8	4(80)	0
Fusobacterium nucleatum	PPT	≤8	≤8	≤8	≤8	0	0
2	CFO	≤4	≤4	≤4	≤4	0	0
	IMI	≤1	≤1	≤1	≤1	0	0
	CLI	≤0,5	≤0,5	≤0,5	≤0,5	0	0
	MET	≤2	4	≤2	4	0	0
	PEN	≤0,125	≥8	≤0,125	≥8	1(50)	0
Propionebacterium acnes	PPT	≤8	≤8	≤8	≤8	0	0
1	CFO	≤4	≤4	≤4	≤4	0	0
	IMI	≤1	≤1	≤1	≤1	0	0
	CLI	≤0,5	≤0,5	≤0,5	≤0,5	0	0
	MET	≥128	≥128	≥128	≥128	1(100)	0
	PEN	≤0,125	≤0,125	≤0,125	≤0,125	0	0

Legend: MIC: Minimum Inhibitory Concentration; PPT: Piperacillin+Tazobactam; CFO: Cefoxitin; IMI:Imipenem; CLI: Clindamicyn; MET: Metronidazole; PEN: Penicillin.

Table 4. Levels of inflammatory cytokines in the abdominal secretion and in the serum of evaluated patients

Patient	Cytokines in the abdominal secretion (pg/mL)						Cytokines in the serum (pg/mL)							Recovered microorganisms
	IL-8	IL-1	IL-6	IL-10	TNF	IL-12P70	Patient	IL-8	IL-1	IL-6	IL-10	TNF	IL-12P70	
1	1084212.7	5100032.3	0.0	0.0	0.0	257.0	**1**	*						*Proteus mirabilis/ Sphingomonas paucimobilis/ Streptococcus anginosus*
2	1813294.1	170102.6	515.5	0.0	0.0	236.0	**2**	*						*Escherichia coli/P. mirabilis/B.fragilis*
3							**3**	*						No growth was observed
4	4045.6	0.0	3538.8	0.0	0.0	228.9	**4**	1524.0	1.7	28.8	3.5	0.0	9.4	No growth was observed
5	310529.5	3219.3	0.0	0.0	0.0	0.0	**5**							No growth was observed
6	77736.7	0.0	0.0	0.0	0.0	170.3	**6**	19.6	1.9	48.3	0.0	10.9	0.0	No growth was observed
7	316960.3	11996.2	0.0	0.0	0.0	0.0	**7**	64.1	0.0	95.7	8.4	0.0	8.4	No growth was observed
8	2142880.2	17518.5	876733.8	1857.8	478.5	214.8	**8**	*						*Acinetobacter baumannii/S. epidermidis/ Candida kefyr*
9	*						**9**	*						*Streptococcus pluranimalium/ Fusobacterium nucleatum*
10	1438673.7	340713.6	0.0	0.0	0.0	0.0	**10**	*						*Kocuria kristinae/ Prevotella bivia/ F. nucleatum*
11	*						**11**	69.0	0.0	88.9	0.0	7.4	5.8	No growth was observed
12	777651.0	465369.4	0.0	0.0	5266	313.3	**12**	*						*Streptococcus constellatus*
13	122693.6	965.2	0.0	0.0	0.0	0.0	**13**	*						No growth was observed
14	*****						**14**	*						*Gemella morbillorum/ Prevotella intermedia*
15	190679.8	24362.2	42256.6	0.0	298.8	342.0	**15**	*						No growth was observed
16	753241.6	124084.5	0.0	0.0	0.0	228.7	**16**	*						*Staphylococcus epidermidis*
17	*						**17**	3075.5	23.5	63879.7	15.1	0.0	6.6	*S. anginosus /Pediococcus pentosaceus/Streptococcus dysgalactiae/ S. paucimobilis*
18	*						**18**	1739.5	0.3	88.3	0.0	0.5	4.6	*Staphylococcus warneri*
19	*						**19**	2850.5	7.7	40534.2	479.3	0.5	7.1	*Hafnia alvei/E. faecalis*
20	1503196.1	243553.0	8048.5	0.0	510.5	250.2	**20**	13.5	1.4	384.9	5.4	0.8	4.9	*E. coli/S. epidermidis/ Bacteroides ovatus*
21	277497.0	28828.3	0,0	0.0	235.3	0.0	**21**	*						*E. coli/Enterococcus faecalis*
22	3868682.7	565058.1	0.0	0.0	0.0	0.0	**22**	*						*E. coli/ B. fragilis*
23	73989.8	37772.4	537.1	0.0	0.0	0.0	**23**	46.6	2.8	350.3	19.0	6.9	7.9	*Bacteroides vulgatus*
24	*						**24**	883.2	5.2	12579.2	163.6	0.0	6.8	No growth was observed
25	1503196.1	839445.3	360145.7	260.3	39299.5	193.3	**25**	*						No growth was observed
26	*						**26**	250.4	7.6	5062.7	22.4	0.0	4.9	*Acinetobacter baumannii/ C. freundii/ Staphylococcus aureus*
27	112178.2	1406.3	1820.3	0.0	0.0	154.9	**27**	552.8	3.6	1644.7	11.0	0.0	8.1	No growth was observed

Patient	Cytokines in the abdominal secretion (pg/mL)						Cytokines in the serum (pg/mL)							Recovered microorganisms
	IL-8	IL-1	IL-6	IL-10	TNF	IL-12P70	Patient	IL-8	IL-1	IL-6	IL-10	TNF	IL-12P70	
28	*						**28**	0.0	0.0	0.0	93.5	0.0	183.0	No growth was observed
29	*						**29**	2357.5	41.6	17240.4	0.0	0.0	0.0	*P. bivia/P. melaninogenica*
30	*						**30**	*						No growth was observed
31	684720.1	250814.9	362.4	0.0	2245.2	193.3	**31**	*						*Providencia rettgeri/Citrobacter freundii*
32	*						**32**	65.7	14.1	622.1	51.8	72.3	9.5	No growth was observed
33	*						**33**	3.6	0.0	7.3	0.0	0.0	0.0	No growth was observed
34	777651.0	143144.5	0.0	0.0	0.0	154.9	**34**	13.1	0.9	61.2	0.0	0.0	0.0	*P. pentosaceus/S. paucimobilis/E. faecalis/ B. fragilis/ Bacteroides thetaiotaomicron*
35	*						**35**	1291.5	1.9	22825.5	18.7	0.3	6.1	*E. coli/E. faecalis/S. epidermidis*
36	*						**36**	*						*S.aureus/Providencia stuartti/K. kristinae/A.baumannii/ Candida albicans*
37	*						**37**	112.6	2.1	94.6	6.7	0.0	9.6	*Pseudomonas aeruginosa/P. stuartii/ Staphylococcus haemolyticus/ P. bivia*
38	130259.0	2434.5	21505.4	0.0	0.0	137.3	**38**	*						No growth was observed
39	*						**39**	1978.3	25.3	6361.3	156.5	0.0	6.4	*S. haemolyticus/A. baumannii/Enterococcus faecium/Pseudomonas spp./Candida albicans*
40	*						**40**	0.0	0.0	0.0	333.3	0.0	189.5	*Streptococcus sanguinis/S. constellatus/ G. morbillorum/Morganella morganii/ S. pluranimalium*
41	*						**41**	111.8	0.0	61.7	2.5	0.4	5.1	No growth was observed
42	450193.6	4399.0	29465.0	0.0	679.0	271.0	**42**	112.5	0.0	50.6	0.0	0.2	5.8	*E. faecium/A. baumannii*
43	103604.9	1652.6	0.0	0.0	306.9	285.0	**43**	4.4	0.0	9,4	0.0	0.0	6.9	No growth was observed
44							**44**	207.1	0.5	2231.2	0.2	0.5	3.6	*S. warneri*
45	1275791.6	75290.2	490670.4	0.0	1258.1	0.0	**45**	427.0	0.5	204.1	0.8	0.0	3.5	*E. coli/E. faecalis*
46	258389.6	139108.9	740.6	0.0	0.0	163.0	**46**	64.1	0.5	31.0	6.8	0.2	4.2	*S. epidermidis*
47	699348.5	65132.4	38294.3	00	0.0	163.0	**47**	67.0	0.0	26.1	0.0	0.1	4.4	*Propionibacterium acnes*
48	1248318.0	63629.0	6735.2	0.0	559.3	0.0	**48**	74.2	0.3	47.9	0.1	0.5	6.8	*P. pentosaceus/ P. mirabilis/ E. faecalis*
49	147594.1	156018.3	0.0	0.0	306.9	299.2	**49**	7.1	2.7	26.9	1.1	1.2	1.8	*Bacteroides fragilis*
50	427422.8	33156.2	1456.9	0.0	0.0	423.7	**50**	8.4	1.5	11.1	0.9	1.5	1.7	*S. anginosus/ S. pluranimalium/ S. paucimobilis/ B. fragilis*
51	210937.8	18808.1	0.0	0.0	0.0	214.8	**51**	*						*G. morbilorum/S. paucimobilis*

** Insufficient samples for this analysis*

Despite the available treatment options for MRSA infections, unfortunately the morbidity and mortality associated to this group of pathogens remain high [26]. In the present study, two samples of *S. aureus* and seven of nine samples of coagulase-negative *Staphylococcus* were resistant to oxacillin/cefoxitin. Of note, the most important obstacle in the treatment of staphylococcal infections is methicillin resistance because they are also resistant to others beta-lactam agents [26,27].

Studies have shown that infections caused by vancomycin resistant *Enterococcus* (VRE) are associated with a higher mortality rate than is seen for vancomycin-sensitive *Enterococcus*. Resistance to new antimicrobial agents like daptomycin and linezolid has been also described [28]. One sample of *E. faecium* was resistant to a wide range of antibiotics, including vancomycin.

We found resistance to penicillin G in 80% of anaerobic strains, reaching 100% in *B. fragilis* group, which is assumed to be highly resistant to most anaerobic antimicrobial agents [22,29,30]. We found that 80% of all anaerobes isolated were ESBL producers and association of β-lactamase inhibitor proved to be very effective when combined to β-lactam agents, like piperacillin and tazobactam. ESBL is indeed a common mechanism of anaerobes resistance, in a study by Wybo et al. [31], 52% of the anaerobes isolates were ESBL positives, especially *Bacteroides* which resistance reached up to 96% of the samples. In our study, 87.5% of the *Bacteroides* were ESBL positive. These findings reinforces that IAI associated to *B. fragilis* should not be treated with single therapeutic agent. We found that interesting therapeutic options for anaerobes are clindamycin and cefoxitin, since most of the samples were sensitive to them, 73% and 80% respectively, result similar to those described by Wybo et al. [31]. It has been observed that cefoxitin has shown good efficacy against anaerobes, with sensitivity rates relatively stable, 83% in 1993-1994 and 79% in 2011-2012 [31].

According to the literature, even after 45 years use, metronidazole remains the drug of choice for the treatment of anaerobic infections [13,32]. In this study, this was the main antimicrobial utilized empirically to patient treatment. The only microorganism resistant to metronidazole was *P. acnes* for which the resistance is intrinsic, as reported by CLSI (2015) [16] and by Santos et al. [22]. Imipenem showed 100% effectiveness against the anaerobes GNR, being active also against *P. acnes*. Similar findings, close to 100% sensitivity to metronidazole, imipenem or piperacillin+tazobactam were observed by Karlowsky et al. [33], in a study conducted in Canada during the years 2010 and 2011.

IAI are associated with strong inflammatory processes, which can extend systemically. To gain insight about how inflammation was being modulated by the therapy or infection origin, we measured inflammatory (IL8, IL1, IL6, TNF and IL12) and regulatory (IL10) cytokines in abdominal secretion or serum of patients with IAI. We observed higher proinflammatory profile in the abdominal secretion with higher levels of IL1, IL8, and IL12 when compared to serum levels. High serum levels of cytokines are a sign of severe infections and associates with bad outcome [24,34]. Since inflammation is triggered directly by infection, not surprisingly, abdominal secretions in which bacteria were isolated displayed higher levels of inflammatory cytokines than culture negative samples. This result supports that correct choice of antibiotic therapy early during infection is important to prevent not only bacterial over growth, but also uncontrolled inflammation. Likewise, nosocomial infections displayed a trend to higher inflammatory profile than community-acquired infection, suggesting more aggressive infection, which should be treated with more aggressive therapy. Accordingly, Barnett et al. [35] also observed that nosocomial-acquired infections are more severe, requiring longer hospitalization and showing higher death rates.

On the other hand, IL10 displayed higher systemic levels suggesting a mechanism to counter regulate systemic inflammation. IL10 is an important anti-inflammatory cytokine essential to regulate devastating effects of uncontrolled inflammation [36]. On the hand, IL10 can shut down several effector mechanisms of macrophages and T cells that promote infection control [37]. Of note, IL10 was undetectable in 28 from 30 samples in abdominal secretions, perhaps to allow vigorous local inflammation, but it was detected in most of serum samples. Interestingly, high levels of serum IL10 was associated to uncontrolled systemic inflammation and high mortality maybe as a means to control systemic inflammatory syndrome during a bad infection evolution [38].

Many were the limitations of this study that should be consider for the interpretation of the results and a new eventual experimental proposal among them, the deficiencies in access to all data of all patients, the absence of serum and secretions of some patients and the impossibility to monitoring of patients over time.

5. CONCLUSION

Our data supports the necessity of performing the prevalence and antimicrobial susceptibility profile analyzes in cases of IAI, if not in the clinical routine, at least periodically, taking into account the great diversity of microorganisms recovered, the virulence and multiresistant profile observed specially among those of hospital origin and, indicates the importance of further evaluation of cytokine profile in IIA for a better understanding of the evolution of these infections.

DISCLOSURE STATEMENT

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors declare that there is no conflict of interest.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14309