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Mini review

Correlation between antibiotic resistance and clinical outcome of anaerobic infections; mini-review

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ABSTRACT

In anaerobic infections, the relationship between clinical failure and antibiotic resistance is difficult to demonstrate, especially in mixed anaerobic-aerobic infections. Single isolates of anaerobes in cases of bacteraemia revealed that treatment failures were due to inappropriate therapy. We review here cases, where the empiric treatment was unsuccessful due to resistance of anaerobic bacteria to the administered agents and where the change of the antibiotic allowed the patients to be cured. Many therapeutic failures could be linked to the lack of timely detection of resistance, including heteroresistance of the anaerobes. Disk diffusion or Etest methodology may be suitable, at least for rapidly growing anaerobes, to detect both resistance and heteroresistance to antibiotics widely used for empirical therapy.

1. Introduction

Many factors may play an important role in the healing of anaerobic or mixed infections, including bacterial synergism, inoculum effect, time to treatment initiation, antibiotic concentration at the infection site, the underlying medical condition of the patient, to mention a few [1]. When surgical procedures such as abscess excision or drainage, tissue debridement are combined with antibiotics or even hyperbaric oxygen therapy, it is difficult to decide the precise role of antibiotics in the cure of the infection. Following surgery, some patients respond favorably without the administration of antibiotics or even with usage of an inactive antibiotic against anaerobes. While on the other hand, if surgery is not performed, curing of anaerobic infections may not be achieved even with application of appropriate antibiotic therapy. The correlation between *in vitro* susceptibility test results, obtained by different testing methods, and clinical response is difficult to be established in anaerobic infections [2]. Many studies have shown that antibiotics, which are active against anaerobes provide higher success rates, including lower mortality rates in anaerobic sepsis, than those lacking activity against these organisms [3]. However, there are also contradictory opinions whether antibiotic susceptibility testing of anaerobes improve the cure rate of the associated infection [4–7]. Some agents like metronidazole, β-lactam + β-lactamase inhibitor combinations, chloramphenicol, or carbapenems allow clinicians to efficiently treat the majority of anaerobic infections without knowing the susceptibility of the causative agents. With the widespread use of genetic method for the detection and identification of anaerobes, antibiotic susceptibility testing is often not carried out to provide data for the change of empiric therapy. In case of treatment failure or in the specific clinical situations mentioned above, determination of antibiotic susceptibility of the isolated anaerobes may be unavoidable, so that empirical treatment can be adjusted accordingly.

2. Paradigms in the second part of the XXth century

We must remember the old discussions between bacteriologists and clinicians over the interest of determining the antibiotic susceptibility of anaerobes. Chow et al. [4] claimed that appropriate antibiotic therapy of *Bacteroides* bacteremia is associated with a

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better outcome than inappropriate therapy, but some surgeons advocated, evaluating retrospectively the effect of empiric antibiotic therapy in 200 patients [5], that "routine practice of obtaining peritoneal cultures in patients operated upon for acute and complicated appendicitis should be abandoned". Mosdell et al. [6] evaluated retrospectively the clinical outcome of 480 patients with secondary peritonitis. They observed that surgeons typically ignore culture data after operation. There were only 41 of 480 (8.5%) patients, whose empirically selected antibiotic treatment was successfully changed, based on the culture results and susceptibility testing. It was questioned whether obtaining intraoperative cultures may benefit patients' outcome. In a similar study, Dougherty et al. [7] found that in the case of 104 patients with appendicitis involving aerobic and anaerobic bacteria, culture results appeared to influence the antibiotic therapy only in 7 of 104 (6.7%) patients. In another study on 175 patients with intra-abdominal infection, the susceptibility of both aerobic and anaerobic bacteria was statistically correlated with outcome [8]. Of the anaerobes, isolated from intraoperative cultures, Hopkins et al. [8] found that 5 of 19 (26%) *B. fragilis* strains were resistant obtained from those who had a postoperative infection, *versus* only 1 of 37 (3%) patients without postoperative infection had resistant isolate. Among the 131 patients who recovered from intra-abdominal infections, 57 (44%) had resistant isolates (both aerobic and anaerobic) to the antibiotics used in the therapy, whereas 82% of the patients (36 of 44) with complications harbored resistant isolates in the intraoperative culture [8]. Snydman et al. [2] emphasized that there was limited information regarding the correlation of clinical outcomes in patients with *B. fragilis* group infections and the susceptibility data. In their retrospective study they found that the most important predicting factor for favorable outcome was the time during which the cefoxitin concentration was above MICs against the *B. fragilis* isolates.

At this point of the debate, Wilson et al. [3] stated that there is a worrisome difference between the viewpoints of the surgeons and microbiologists concerning the usefulness of the bacteriological data (including species identification and antibiotic susceptibility testing of the anaerobes) in treatment of mixed intraabdominal infection. They suggested that successful antimicrobial therapy should be based on the susceptibility of the microbiota of the operative site. They also emphasized that the culture of the specimens from intraabdominal infections and antimicrobial susceptibility testing may help to change antimicrobial treatment in patients who have resistant organisms (including anaerobes); thus providing a better clinical outcome.

3. Focus on anaerobic bacteremia

All previous studies referred on mixed infections involving aerobe and anaerobe species, such as intra-abdominal infections connected with surgery, where many factors may interfere with the clinical outcome, beside the choice of the proper antibiotic against the anaerobic part of the mixed microbiota. More information can be obtained in anaerobic bacteremia cases, where a single anaerobic strain is isolated from the blood culture. Salonen et al. [9] evaluated 57 patients who had clinically relevant anaerobic bacteremia. Twenty eight of 57 (49%) patients received effective antibiotic treatment from the beginning and only 5 of 28 (18%) patients died. In the group where the initial treatment was ineffective (18 patients), but therapy has been changed based on susceptibility results, only 3 of 18 (17%) patients died. The initial treatment of the anaerobic bacteremia was started with an ineffective drug and the therapy was not changed in the case of 11 patients (19%), in this group 6 of 11 patients died (55%). In this well-designed retrospective study on patients with anaerobic bacteremia, the difference in the mortality rates between the patients who received ineffective treatment and the groups with either susceptible isolates to the originally selected antibiotic or with change of the therapy according to the resistance data, was statistically significant [9].

A similar study on anaerobic bacteremia was done by Kim et al. [10] evaluating the blood culture isolates of 70 non-duplicate anaerobic bacteremia patients. They concluded that the survival rate of anaerobic bacteremia was significantly worse (82% *versus* 76%) in patients who received inappropriate therapy compared with those who underwent appropriate therapy based on antibiotic resistance determination. The most frequently isolated organisms were *B. fragilis* and *B. fragilis* group species (50 isolates), *Clostridium* spp. (9 isolates) as well as other clinically relevant anaerobes (11 isolates).

During a prospective multicenter observational study Nguyen et al. [11] demonstrated that *in vitro* activity of agents in case of *Bacteroides* species reliably predicts clinical outcome: the specificity was 97%, and the positive predictive value was 82%. Altogether 128 bacteremic patients were involved. The mortality rate, for patients who received therapy to which the *Bacteroides* blood culture isolate was resistant *in vitro*, was significantly higher (45%) than the mortality rate for those who received therapy to which the isolate was susceptible (16%). When failure for clindamycin or piperacillin therapy occurred, clindamycin MIC was 16–256 mg/L and piperacillin MIC was 256 mg/L, respectively for the *Bacteroides* blood culture isolates. It has been concluded that it is advisable to carry out antimicrobial susceptibility testing for blood culture isolates belonging to *Bacteroides* genus.

4. Improved detection of antibiotic resistance of anaerobic bacteria allows to find more clinical failures associated to their resistance

Surveys of antimicrobial susceptibility of anaerobes shows an increase in resistance to various antibiotics regularly used for empiric therapy in mixed infections. It has particularly been observed among the *B. fragilis* group isolates and *Prevotella* spp. [12–16]. Clinical failures have been described in metronidazole-treated patients harboring metronidazole resistant strains [17,18], as well as in the presence of heteroresistant strains [19,21].

To provide more arguments that antibiotic susceptibility testing of anaerobes may be lifesaving in some instances, we have selected published clinical cases, where patients treated empirically for monobacterial or mixed infection have experienced clinical failure; this failure associated with *in vitro* resistance data of *Bacteroides*/*Prevotella* strains tested on the following days to the given antibiotics. The definitive proof is that most of the patient has been cured by reconsidering the empiric treatment (Table 1). According to the case reports all treatments had been given at adequate dosage regimen. In all three cases reported by Rotimi et al. [18] patients had been treated by a combination of cephalosporines (2nd or 3rd generation) with metronidazole. High level resistance of *Bacteroides* species to metronidazole (MIC >32 mg/L) was observed in all three cases. Changing of the empiric antibiotic therapy to imipenem (case 1) or amoxicillin-clavulanic acid (case 2), based on the *in vitro* susceptibility results of *B. fragilis* and *B. ovatus* isolates led to recovery of the patients showing that metronidazole resistance was responsible for clinical failures in both cases. While in case 3 multidrug-resistant aerobic bacteria (*Pseudomonas aeruginosa* and *Enterobacterales* spp.) were also present beside the multidrug-resistant *Parabacteroides (Bacteroides) distasonis* resistant to metronidazole, carbapenems, clindamycin and piperacillin/tazobactam [18].

Within the *B. fragilis* group, decreased susceptibility to

Table 1
Monobacterial and polymicrobial infections where the metronidazole resistance of *Bacteroides/Prevotella* sp. was suspected as the reason of clinical failure.

Cases (ref.)	Microorganism	Empiric treatment	Antimicrobial susceptibility results of the anaerobic species[e]	Antibiotic active against the anaerobic species and used for targeted therapy	Outcome
1 [19]	*B. fragilis*	ceftazidime 2g bid + metronidazole 500 mg tid	CTZ-R, MET-R (MIC >32 mg/L)	imipenem 500 mg tid	Recovered
2 [19] [a]	*B. fragilis* and *B. ovatus*	cefuroxime 750 mg tid + metronidazole 500 mg tid	CUR -R MET-R (MIC >32 mg/L)[f]	amoxicillin-clavulanic acid 600 mg tid	Recovered
3 [19] [b]	*P. (B.) distasonis*	ceftriaxone 2g + amikacin 500 mg once a day + metronidazole 500 mg tid	CTR-R AMI-R MET-R (MIC >32 mg/L)	meropenem 1g tid + amikacin + cefepim	Died
4 [20] [c]	*B. fragilis*	cefuroxime 750 mg tid + metronidazole 500 mg tid later: + gentamicin 7 mg kg/day	CUR -R MET-R (MIC: 6 mg/L) GENT-R	meropenem 1g tid	Recovered
5 [21]	*Prevotella loescheii*	chloramphenicol 1h qid + metronidazole 400 mg tid	ND MET-R (MIC: 12 mg/L)	chloramphenicol 1g qid + clindamycin 600 mg bid	Recovered
6 [22] [d]	*Prevotella* spp.	cefotaxime 1g tid + ofloxacin 200 mg later: teicoplanin 400 mg + metronidazole 500 mg tid	CTA-R OFL-R TEI-R MET-R (MIC: 64 mg/L)	piperacillin-tazobactam 4g tid	Recovered

[a] Presence of *E. coli* and *P. aeruginosa* susceptible to cefuroxime.
[b] Presence of multiresistant *P. aeruginosa* and *Enterobacterales* spp.
[c] Presence of *Enterococcus faecalis* susceptible to gentamicin and cefuroxime.
[d] Presence of *E. coli* susceptible to cefotaxime and ofloxacin and *S. anginosus* susceptible to cefotaxime.
[e] : Suggested abbreviation of antibiotics by the EUCAST System.
[f] : Both *B. fragilis* and *B. ovatus* had the same resistance level to metronidazole.

metronidazole (MIC 8–16 mg/L) may also lead to failure, (being the sensitivity breakpoint 4 mg/L). Elsaghier et al. [19] reported the case of a 70-year-old man who was admitted for excision of rectal adenoma. Empirical treatment with intravenous cefuroxime (750 mg/8 h) in combination with metronidazole (500 mg/8 h) was started. Three days after surgery, the patient developed signs of peritonitis. From the specimens taken during surgery mixed coliforms grew, susceptible to cefuroxime, *B. fragilis* susceptible to a 5 μg metronidazole disk and *Enterococcus faecalis*. Four days later the patient did not improve and gentamicin 7 mg/kg once daily was added to the therapy. *B. fragilis* was isolated from blood culture bottle; the strain was susceptible by disk diffusion to amoxicillin-clavulanate, clindamycin and meropenem, but resistant to a 5 μg metronidazole disk (MIC 6 mg/L by Etest). Cefuroxime and metronidazole were stopped and treatment was changed to meropenem (1 g/8 h). The patient became apyrexial and was discharged home 12 days later. Colonies within the metronidazole inhibition zone with a 5 μg disk were observed (sign of heteroresistance). No *nim* genes were detected by PCR.

Sandoe et al. [20] reported a clinical failure due to a metronidazole-resistant *Prevotella loescheii.* A 62-year-old man, with a 2-day history of headache, vomiting and confusion due to a subdural empyema, received an empirical treatment by meropenem, 1 g every 6 h. At 48h the anaerobic cultures from the pus became positive and antimicrobial therapy was changed to chloramphenicol (1 g every 6 h) and metronidazole (500 mg every 8 h). Colonies within the metronidazole inhibition zone (5 μg disk) appeared. The metronidazole MIC determined by Etest was 12 mg/L. No *nim* genes were detected. Metronidazole was changed to clindamycin, and chloramphenicol was continued. The patient became apyrexial. The authors stressed that metronidazole heteroresistance in anaerobes may lead to in treatment failures [20].

Mory et al. [21] reported a case of a 76-year-old patient who was hospitalized for fever and altered clinical status and received cefotaxime 1g t.i.d. and ofloxacin 200 mg b.i.d. *Streptococcus anginosus* susceptible to cefotaxime and an *Escherichia coli* strain susceptible to cefotaxime and of loxacine were isolated. On day 7 the patient remained febrile. A strictly anaerobic gram-negative rod was detected in the blood culture: *Prevotella* spp. Small colonies grew inside the metronidazole disk (5 μg/disk) inhibition zone after 72h incubation time. Metronidazole MICs of 2 and 64 mg/L were found for colonies outside and within the inhibition zone, respectively. On day 14 the treatment was changed for piperacillin-tazobactam 4 g every 8 h and the patient became apyrexial. No *nim* genes were detected by PCR techniques.

Beside *Bacteroides* and *Prevotella* isolates resistant to metronidazole, other anaerobic species may cause treatment failure due to resistance to the selected therapy. Hidri et al. [22] reported about a 56-year-old man who was hospitalized for femoro-popliteal bypass followed by wound infection at the site of operation. *Enterobacter cloacae* (a hyperproducer of a cephalosporinase) and *Proteus mirabilis* (wild type) were isolated from the abscess. After treatment with imipenem and ciprofloxacin, pyrexia persisted and *Egerthella lenta* was isolated from the blood cultures identified by 16S rRNA sequencing. Using a disk diffusion method, a double zone of inhibition was observed around the imipenem disk. MIC values (0.38 mg/L and >32 mg/L) were found for the imipenem susceptible and resistant subpopulations, respectively. No β-lactamase production was detected using the nitrocefin test. The patient was treated with metronidazole during the next 14 days and recovered.

Metronidazole heteroresistance in *Clostridioides difficile* was found in 9 out of 73 patients by Pelaez et al. [23]. Regardless of using metronidazole Etest or 5 μg disk, colonies appeared in the inhibition zone after 5 days of incubation in anaerobic environment. Relapses may occur during *C. difficile* infections (CDI) treated by any adequate antibiotic, thus the impact of possible resistance to

metronidazole on clinical failure in *C. difficile* cases is difficult to evaluate. In a Spanish study, 10 of 64 (15.6%) patients who had metronidazole susceptible strain had ≥1 recurrences, while 4 of 9 (44%) patients with metronidazole heteroresistant CDI experienced ≥1 recurrences [24]. Heteroresistance for cefoxitin and carbapenems has been described for different species of *Bacteroides* genus too, isolated from clinical samples or from feces [25–27]. In such cases Etest could reveal and confirm the heteroresistance which may influence the empirical usage of these antibiotics. Like in some aerobic species, such as *Acinetobacter baumanii*, the detection of heteroresistance to imipenem may guide empiric therapy and prevent clinical failure [28,29]. In addition, beta-lactamase producing anaerobes in mixed infections allow the survival of antibiotic susceptible coinfecting pathogens [30]. Heteroresistance is an important phenotype clinically and its detection in the routine microbiology practice, both in anaerobes and aerobes, can be important.

5. Conclusion and prospective

The data show that severe mixed infections involving anaerobes not treated appropriately could result in unfavorable clinical outcomes. Therefore, clinicians should publish studies about failure of empirical treatment of anaerobic mixed or monobacterial infections and the advantage to consider antibiotic resistance patterns of the anaerobic bacteria during therapy. In addition, microbiologists should attempt to develop timely and easier susceptibility testing methods for the anaerobes. The disk diffusion technique was not accepted as a reference method for anaerobes [31], but should be reevaluated [32,33]. First of all, by introducing the concept of “areas of technical uncertainty”, it is possible to obtain “very major error” rate as low as 1.4% [31]. Secondly, unlike the dilution methods, the disk diffusion method similarly to Etest methodology allow to demonstrate easily the double zone around the disk or presence of tiny colonies within the inhibition zone as the sign of the heteroresistance. The same has been shown by several studies in the case of different isolates of *Bacteroides* spp [25–27]. EUCAST together with ESGAI (ESCMID Study Group on Anaerobic Infections) are actually working on developing methods and rules for future using of disk diffusion method for anaerobes [33]. Detection of heterogeneous resistance merits to be investigated also with the resurgence of use the disk diffusion method in anaerobe bacteriology. Double zones of inhibition are frequent with β-lactams and metronidazole. Heteroresistance may occur after 72h incubation time. It is therefore necessary to further incubate the plates in anaerobic environment, if small colonies appeared inside the inhibition zone. In the case of MIC determination by the Etest, it is useful to subculture colonies inside the inhibitory zone for further investigation. Sampling and the appropriate transport of the specimen are highly important, as in some occasions, anaerobes are already non-viable during the sample storage or transport and some clinical failures may be due to the lack of successful isolation of the anaerobes.

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Declaration of competing interest

We declare no conflict of interests.

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