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Antimicrobial susceptibility variation of 50 anaerobic periopathogens in aggressive periodontitis: an interindividual variability study

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Background/aims: The frequent use of antibiotics in developed countries has led to the emergence of widespread bacterial resistance. In this study, the interindividual variability of the antibiotic susceptibility of 50 putative microorganisms in aggressive periodontitis patients has been evaluated by means of VC (variation coefficient).
Material and methods: A total of 60 microbial samples were collected from 20 adult patients diagnosed with aggressive periodontitis (2–4 samples by patient). Bacterial strains of *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum,* and *Peptostreptococcus micros* were isolated according to Slots' rapid identification method. The susceptibilities to 10 antibiotics were studied: penicillin G (PEN), ampicillin (AMP), amoxicillin (AMX), amoxicillin/clavulanate (AMC), tetracycline (TET), doxycycline (DOX), ciprofloxacin (CIP), erythromycin (ERY), spiramycin (SPI) and clindamycin (CLIN), using the Disk Diffusion Susceptibility test (DDS test: Kirby-Bauer's modified method for anaerobic bacteria). The broth microdilution Minimum Inhibitory Concentration test was carried out as a control test.
Results: Among the 50 identified bacteria, 15 were *P. gingivalis,* 12 *P. intermedia,* 8 *T. forsythia,* 9 *F. nucleatum,* and 6 *P. micros*. The results of the DDS test show that penicillins (especially AMC, AMP, and AMX), cyclines (especially DOX) and CLIN are highly effective against the 50 anaerobic studied bacteria. CIP and ERY have the lowest efficacy against those bacteria. CIP shows a very variable activity according to anaerobic bacteria species, being particularly inactive against *P. gingivalis* and very efficient against *T. forsythia* and *P. micros*. SPI is also highly efficient but not against *P. micros*.
Conclusions: The interindividual susceptibility of principal periodontal pathogens to antibiotics is not homogeneous and seems to vary according to bacterial species and antimicrobial molecules. This variability seems to be greater with older molecules (PEN, TET, ERY) than with more recent ones, which indicates more stable results (AMC, AMX, AMP, and DOX). *P. intermedia* appeared to be the bacteria most resistant to penicillins and showed the highest coefficient variation. Together with scaling and root planing, the combination of two antibiotics would therefore seem to be recommended in the treatment of aggressive periodontitis, particularly in the presence of *P. intermedia*.

Key words: aggressive periodontitis; antibiotics; antimicrobial susceptibility testing; *Fusobacterium nucleatum*; *Peptostreptococcus micros*; *Porphyromonas gingivalis*; *Prevotella intermedia*; *Tannerella forsythia*; variability; variation coefficient

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Advanced periodontal disease is believed to be an opportunistic infection caused by subgingival indigenous anaerobic and capnophilic bacteria of dental plaque (44, 60). Some patients may respond poorly to the conventional periodontal therapy, which includes the suppression of the putative microorganisms by mechanical or surgical means. In these patients, additional systemic antibiotic treatment can improve the effect of mechanical debridement (54, 68, 76).

Amoxicillin is very frequently prescribed by periodontists (58, 72). But, as the enzymatic activity of beta-lactamase has the capacity to inactivate penicillin, ampicillin, and amoxicillin (69, 74), the amoxicillin/clavulanate combination is becoming more commonly used (16, 38), especially against *Prevotella* species and *Porphyromonas gingivalis* (27). Other antibiotics are currently prescribed by periodontists: tetracycline, doxycycline, minocycline, ciprofloxacin and clindamycin (1, 2, 35, 36, 37, 66).

According to Walker (71), 14–36% of the bacterial species isolated from adult periodontitis patients were resistant to one or more of the seven tested antibiotics. Moreover, Kleinfelder et al. (26) showed that 3–29% of the 214 investigated microorganisms were not sensitive to antibiotics. As this sensitivity seems extremely variable depending on species, and almost certainly on antibiotics (39), it was felt that it deserved further in-depth research.

Purpose

Frequent use of antibiotics has unfortunately led to the emergence of widespread bacterial resistance (3, 11, 18, 26, 47, 70). The aim of this study was to evaluate the interindividual variability of the antibiotic susceptibility of some of the major anaerobic pathogens in aggressive periodontitis. There are several reasons to suspect *Tannerella forsythia* (formerly *Bacteroides forsythus*), *P. gingivalis* (6, 17, 22, 56, 62, 63) and *Prevotella intermedia* (55) to be frank periodontal pathogens in this type of periodontitis. The variability of sensitivity to 10 antibiotics has been determined and discussed. Our investigation compared the variability of this bacterial sensitivity for each antibiotic using the variation coefficient (VC).

For each bacterium studied, the minimal inhibitory concentrations (MIC) of three different antibiotic agents have been evaluated as a control before comparison with the DDS test result.

Material and methods

Patients

Twenty patients with aggressive periodontitis (7, 22) were selected for this study. None of them had used any antibiotics in the previous 6 months or had undergone periodontal mechanical therapy. All were healthy, with no systemic disorders. They were asked to report on smoking habits. Clinical investigations did not include probing depth so as not to cross-contaminate periodontal pockets: subgingival plaque samples were taken from the deepest pockets (> 6 mm) that had been previously determined according to the importance of clinical attachment loss, tooth mobility and radiographically determined bone loss.

Microbiological sampling

After careful removal of supragingival dental plaque, isolation of the sampling sites with cotton rolls and gentle air-drying, two endodontic sterile paper points were consecutively inserted into the pocket for at least 20 s (52). The paper points were then inserted into 2 ml bottles of VGMA III reduced transport medium (Möller (40) modified by Slots (53)), which allows an adequate quantity and quality conservation of subgingival bacterial species for 24–48 h at ambient temperature (4, 40, 52, 53, 65). A total of 60 microbial samples were collected from the 20 patients diagnosed with aggressive periodontitis (2–4 samples per patient).

Microbiological procedures (processing)

To liquefy the VGMA III transport medium, the 2 ml bottles were reheated at 37°C for 15 min. After mixing for 30 s at maximal speed on a Vortex mixer, the 2 ml bottles containing glass beads were opened in an anaerobic chamber (Bactron® IV Anaerobic Environmental Chamber, Sheldon Mfg, Cornelius, OR) and samples were tenfold serially diluted in Wilkins-Chalgren® broth (WC®, Oxoid, Basingstoke, Hampshire, UK).

Appropriate dilutions of 100 µl (10^{-1}, 10^{-2}, and 10^{-3}), were plated on nonselective 5% sterile defibrinated sheep blood agar plates (BioMérieux, Marcy l'Etoile, France) supplemented with 0.0002% menadione sodium bisulfite and 0.4% hemin chloride (Sigma, St. Louis, MO) for total anaerobic bacterial count and cultivation of *P. gingivalis*, *P. intermedia*, *T. forsythia (*previously classified as *Tannerella forsythensis* and *B. forsythus*) (49) and *Peptostreptococcus micros*. In the same way, 100 µl of appropriate dilutions were plated on a selective medium in order to cultivate *Fusobacterium nucleatum* exclusively (CVE medium) (73). Agar plates were then set in the anaerobic chamber for 5 days at 37°C.

Bacterial characterization

Identification of putative anaerobic bacteria was carried out according to Bergey's manual criteria (19) as follows: colonial morphology, colonial long-wave ultraviolet fluorescence, cell mobility and morphology, gram-staining, catalase, and oxidase slide tests. If no definitive identification was made, isolates were characterized by means of the API 32-A system® (BioMérieux, La Balme Les Grottes, France) and aerotolerance.

Antibiograms (susceptibility testing)

At this stage of experimentation, from each agar plate containing well-identified *P. gingivalis*, *P. intermedia*, *T. forsythia*, *F. nucleatum* or *P. micros* bacteria, we seeded several colonies in distinct nonselective 5% sterile defibrinated sheep blood agar plates, which were set under anaerobic conditions. After 48 h of incubation, the agar plates were visually checked to eliminate any that might have been contaminated. A second identification of putative strictly anaerobic bacteria was carried out to make sure that the bacteria were correctly identified.

Disk Diffusion Susceptibility test (DDS test)

The DDS test consists of disks (Sanofi® Diagnostics Pasteur, Marnes La Coquette, France) containing a well-defined concentration of an antimicrobial agent, which can be easily placed directly on the surface of the blood or WC® agar plate formerly seeded with the studied bacterial inoculum (Kirby-Bauer's modified method for anaerobic bacteria). After 24–48 h of incubation under anaerobic conditions, the lowest concentration of drug yielding no bacterial growth *in vitro* could easily be read by measuring the critical diameter (or inhibition diameter). The tests was carried out on 10 antibiotics: penicillin G (PEN), ampicillin (AMP), amoxicillin (AMX), amoxicillin/clavulanate (AMC), tetracycline (TET), doxycycline (DOX), ciprofloxacin (CIP), erythromycin (ERY), spiramycin (SPI) and clindamycin (CLIN).

Broth Microdilution MIC test

This antibiogram method consists in successively inoculating several solutions of WC® broth containing increasing antibiotic concentrations (from 0.25 μg/ml to 128 μg/ml). From the translucency or turbidity of the solution it is possible to evaluate the bacterial growth for each antimicrobial concentration. The MIC (minimum inhibitory concentration) is read as the lowest concentration of antimicrobial agent showing no visible growth of the organism in the solution. In this study, three antibiotics were tested: ampicillin, tetracycline, and erythromycin.

Data analysis

All data were entered independently into PC programs using WORD 2000 for Windows® version 5.03 (Microsoft, Redmond, WA). The WORD® data file was transformed into an EXCEL® file and the respective data subtracted. After data collection, statistical analysis was performed by a biostatistician using SAS® version 6.12.

The assessments were used to calculate the critical diameter mean values (mm) of the 10 antibiotics in relation to the 50 strictly anaerobic bacteria isolated. Student's *t*-test was used for statistical comparisons of bacterial data. Analysis of standard deviation was carried out as well as the determination of *P*-values, which were considered statistically highly significant when $P < 0.001$.

One of the most common processes used in statistics consists in comparing mean values (and the standard deviation (SD) values) of many variables. However, when these values are very different (heterogeneous), another indicator becomes essential. The easiest way to compare the standard deviation of measures whose means are heterogeneous is to divide the standard deviation by the mean size. Shown as a percentage, this coefficient is defined as the variation coefficient (VC): $VC = 100 \times SD/Mean$.

This equation shows and explains the degree of dispersion of a given distribution as a function of the mean value (5). It is in fact simply the standard deviation expressed as a percentage of the average. For example, these two sets of numbers – *1, 2, 3, 4, 5* and *21, 22, 23, 24, 25* – have roughly the same standard deviation but have very different VC (67% and 9%, respectively). However, it is not possible to give a general rule as to whether VC is acceptable (low) or not (high).

Although often forgotten, the VC is definitely of great value in statistical analysis (20).

Results

Demographic data (patient-related variables)

The 20 patients selected for this report all had chemically and mechanically untreated aggressive periodontitis that had been diagnosed by their periodontists. The average age of the patients was 41 years (range: 24–62). Sixty samples were taken, from which 50 well-known anaerobic periopathogens of five species were isolated: 15 *P. gingivalis*, 12 *P. intermedia*, 8 *T. forsythia*, 9 *F. nucleatum*, and 6 *P. micros*.

Concordance of results between the two antibiogram techniques

For the 50 bacteria studied, the determination of the minimal inhibitory concentrations of AMP, TET, and ERY was essentially carried out as a control test. The bacterial susceptibility shows a very high similitude between the DDS test and the MIC test: 50/50 strains (100%) for AMP, as well as for TET and ERY.

As a global result, all the bacteria (100%) showed strictly the same susceptibility between the two techniques. This agreement enabled us to validate the DDS test in this study. Consequently, we decided to use the DDS test to study the interindividual variability for each pathogen bacteria. Recommendations adapted from the Antibiogram Committee's Report of the *French Society of Microbiology* (61) were used as references.

Interindividual bacterial susceptibility and variability to the 10 tested antibiotics

Detailed data concerning bacterial susceptibility and variation coefficients are shown in Tables 1 and 2 and Fig. 1–6.

All the *P*-values in this study were statistically highly significant ($P < 0.001$) (Table 2). However, the standard deviation and critical diameter mean values were extremely heterogeneous within antimicrobial molecules and bacterial species (Fig. 1–6). In this, the interpretation of VC values (range: 4.9–68.5%) for each periopathogen is very helpful (Fig. 1–6).

For the 50 strictly anaerobic bacteria, the most active antibiotics were decreasingly classified as following (S = sensitive bacteria, R = resistant bacteria): AMC (100% S), AMP (98% S), DOX (98% S), AMX (96% S), TET (90% S), and CLIN (86% S). The least active antibiotic was CIP (42% R). Two antibiotics had intermediate scores: PEN (70% S) and SPI (68% S).

The 15 *P. gingivalis* strains showed no resistance (0%) to AMP, AMX, AMC, TET or DOX, 13% resistance to PEN, ERY, SPI, and CLIN, and 73% resistance to CIP (Table 1, Fig. 2).

Although the 12 *P. intermedia* were very sensitive to AMC (100%), AMP (92%), DOX (92%), AMX (83%), TET (83%), and CLIN (83%), they nevertheless remained very resistant to CIP (41.5% R). An intermediate susceptibility had been seen with SPI (67% S), ERY (59% S), and PEN (50% S) (Table 1, Fig. 3).

The 8 *T. forsythia* showed a very high sensitivity (100%) to AMP, AMX, AMC, TET, DOX, and CLIN. However, an intermediate susceptibility had been seen with ERY (50% S), PEN (62.5% S), SPI (62.5% S), and CIP (75% S) (Table 1, Fig. 4).

All of the 9 *F. nucleatum* (100%) were sensitive to AMP, AMX, AMC, and DOX (Table 1, Fig. 5). *F. nucleatum* had a 67% susceptibility to SPI (11% R), CLIN (33% R) and PEN (33% R) but was very resistant to ERY (44.5% R). Likewise, 100% of the 6 *P. micros* were sensitive to PEN, AMP, AMX, AMC, DOX, and CLIN (Table 1, Fig. 6). *P. micros* had an intermediate sensitivity to CIP and TET but was very resistant to ERY and SPI.

Regrouping susceptibilities to antibiotics in three profiles

The antibiotics can be grouped into three with regard to their reaction to the 50 strictly anaerobic bacteria:

- Very active: AMC (100%), AMP (98%), DOX (98%), AMX (96%), TET (90%), and CLIN (86%).
- Fairly active: PEN(70%) and SPI(68%).
- Poorly active: ERY(54%) and CIP(46%).

The most sensitive anaerobic bacteria to AMX, and therefore those that do not justify the use of AMC (amoxicillin/clavulanate) at all, were classified in descending order as follows: *F. nucleatum* (Ømean: 41.39 mm), *P. micros* (38.33 mm), *T. forsythia* (36.88 mm), and *P. gingivalis* (36.60 mm).

Nevertheless, although the 12 *P. intermedia* were sensitive to AMX (29.92 mm), of the 50 anaerobic bacteria tested they showed the least sensitivity to PEN (Ømean: 21.42 mm), AMP (28.50 mm), AMX (29.92 mm), and AMC (34.83 mm). The improvement ob-

Table 1. Strictly anaerobic bacteria data: interindividual bacterial susceptibility (%) to the 10 antibiotics tested

	PEN	AMP	AMX	AMC	TET	DOX	CIP	ERY	SPI	CLIN
P. gingivalis ($n = 15$)										
S	80	100	100	100	100	100	27	87	87	87
IS	7	0	0	0	0	0	0	0	0	0
R	13	0	0	0	0	0	73	13	13	13
P. intermedia ($n = 12$)										
S	50	92	83	100	83	92	41. 5	59	67	83
IS	25	0	8.5	0	0	0	17	8	16.5	0
R	25	8	8.5	0	17	8	41.5	33	16.5	17
T. forsythia ($n = 8$)										
S	62.5	100	100	100	100	100	75	50	62.5	100
IS	37.5	0	0	0	0	0	0	12.5	12.5	0
R	0	0	0	0	0	0	25	37.5	25	0
F. nucleatum ($n = 9$)										
S	67	100	100	100	89	100	44.5	11	67	67
IS	0	0	0	0	0	0	22	44.5	22	0
R	33	0	0	0	11	0	33.5	44.5	11	33
P. micros ($n = 6$)										
S	100	100	100	100	67	100	67	33	33	100
IS	0	0	0	0	0	0	33	0	0	0
R	0	0	0	0	33	0	0	67	67	0
Total mean ($n = 50$)										
S	70	98	96	100	90	98	46	54	68	86
IS	14	0	2	0	0	0	12	12	10	0
R	16	2	2	0	10	2	42	34	22	14

S: Sensitive bacteria, IS: Intermediate Sensitivity & R: Resistant bacteria. According to *French Society of Microbiology* (61).
PEN: penicillin, AMP: ampicillin, AMX: amoxicillin, AMC: amoxicillin/clavulanate, DOX: doxycycline, TET: tetracycline, CIP: ciprofloxacin, ERY: erythromycin, SPI: spiramycin, CLIN: clindamycin.

Table 2. Interindividual susceptibility variability (Student's t-test) of the 50 strictly anaerobic bacteria (15 P. gingivalis, 12 P. intermedia, 8 T. forsythia, 9 F. nucleatum and 6 P. micros)

	PEN	AMP	AMX	AMC	TET	DOX	CIP	ERY	SPI	CLIN
ØMin	6	9	6	24	11	16	6	6	6	6
ØMax	40	42	46	46	53	50	40	51	44	48
ØMean	29.10	34.44	36.20	37.96	30.94	31.82	19.76	26.74	26.38	26.98
SD	11.93	7.36	7.65	5.47	8.80	7.33	10.37	14.25	10.61	11.64
VC%	41.00	21.38	21.13	14.40	28.44	23.05	52.47	53.31	40.23	43.16
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

ØMin: minimum critical diameter. ØMax: maximum critical diameter. ØMean: mean of critical diameter.
SD: Standard Deviation. VC: Variation Coefficient (= 100 × SD/Mean). *P*: Probability > |t|.

tained in critical diameter mean (average) between AMX and AMC (4.91 mm; ratio AMX/AMC: 85.90%) is the most important in this group of pathogens. This means that *P. intermedia* is the anaerobic bacterium that produces the greatest quantity of β-lactamases among the five anaerobic species tested in our study.

Discussion

β-lactamase production

Many investigations that have evaluated β-lactamase producing species in periopathogens have found *Prevotella* sp. to be the most frequently involved species (10, 15, 18, 25, 32, 64, 69). This penicillin resistance of *P. intermedia* is in agreement with findings of Listgarten et al. (35) and Feres et al. (13). In the same way, Kuriyama et al. (30) established that the 11 β-lactam antibiotics that they studied showed excellent antimicrobial activity against *P. gingivalis* but not against black-pigmented *Prevotella* (36% were β-lactamase-producing).

The 50 anaerobic strains tested here remain generally susceptible to AMP and AMX (Ømean 36.20 mm) and do not therefore need a routine AMC prescription (improvement of 1.84 mm; ratio AMX/AMC: 95.36%).

Utility of the variation coefficient (VC)

In this study, the VC (variation coefficient) has been extremely helpful in statistical analysis. It showed the degree of dispersion of the distribution of the critical diameters according to value of the mean. The higher the VC, the more the corresponding antibiotic has a variable activity against the studied bacteria, and therefore the more unpredictable is the *in vivo* bacterial eradication. Conversely, the *in vivo* eradication of a periopathogen by an active antibiotic requires as low a VC as possible.

An inactive or intermediately active antibiotic with a low VC signifies that the resistance is stable and regular: this antibiotic should not be prescribed at all. If an antibiotic has an intermediate activity with a high VC this signifies that the resistance is unstable and irregular: the antibiotic in question could then be prescribed (in association preferably) if there is no better alternative. If an antibiotic is active with a very high VC, this means that the activity is unstable and irregular: this antibiotic may at times be incapable of eradicating the periopathogen.

For example, in the case of *P. gingivalis*, the *P*-values were exactly 0.0001 for CIP, PEN, and AMP. This signifies that the mean values of the critical diameters of these three antibiotics were statistically highly significant: but it does not specify the variability's level of resistance and/or its susceptibility. If we then compare the VC for CIP (68.83%), PEN (35.62%), and AMP (17.51%), we notice that *P. gingivalis* had a very high variability to CIP, a tolerable one to PEN, and a low one to AMP, which then had a constant and regular activity against this anaerobic periopathogen.

New findings and comparison with previously published investigations

The present study investigated and compared the variability rate of susceptibility to 10 antibiotics by means of VC (variation coefficient). We have found no previously published study dealing with the problem of sensitivity variability of periopathogens to antibiotics, although some investigations implied it indirectly (39, 45).

In our investigation, the strictly anaerobic periopathogen that showed the highest variation to antibiotics among the five tested species was *P. intermedia* (mean VC: 39.89%). Conversely, *P. micros*, which has already shown low MIC values and high susceptibility rates to all tested β-lactam antibiotics in a previous report (29), demonstrated the lowest variation (mean VC: 22.74%). *F. nucleatum* (mean VC: 31.62%), *T. forsythia* (mean VC:

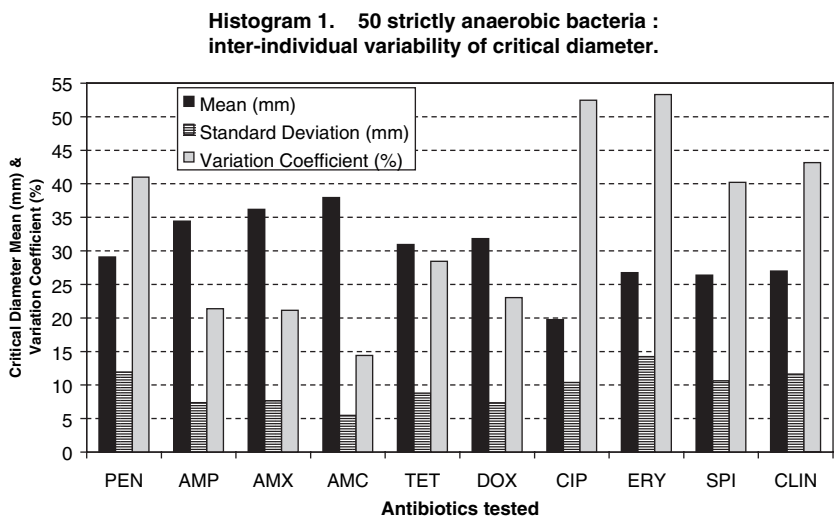

Fig. 1. Histogram of 50 strictly anaerobic bacteria: interindividual variability of critical diameter.

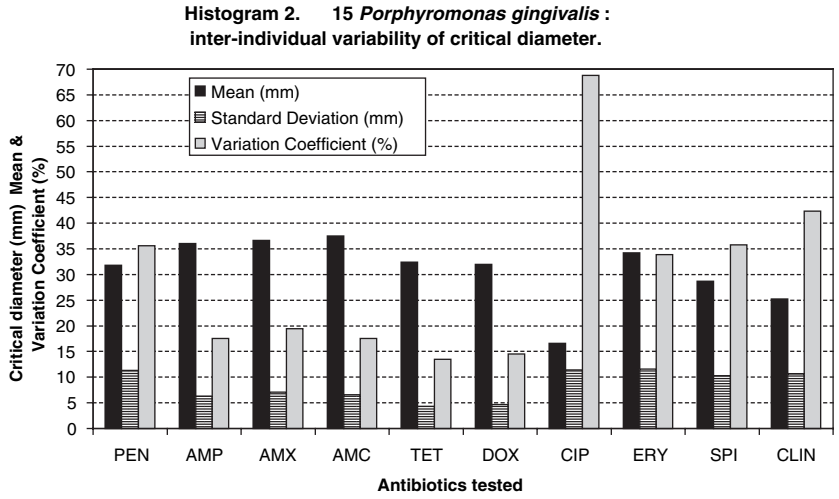

Fig. 2. Histogram of 15 *P. gingivalis* bacteria: interindividual variability of critical diameter.

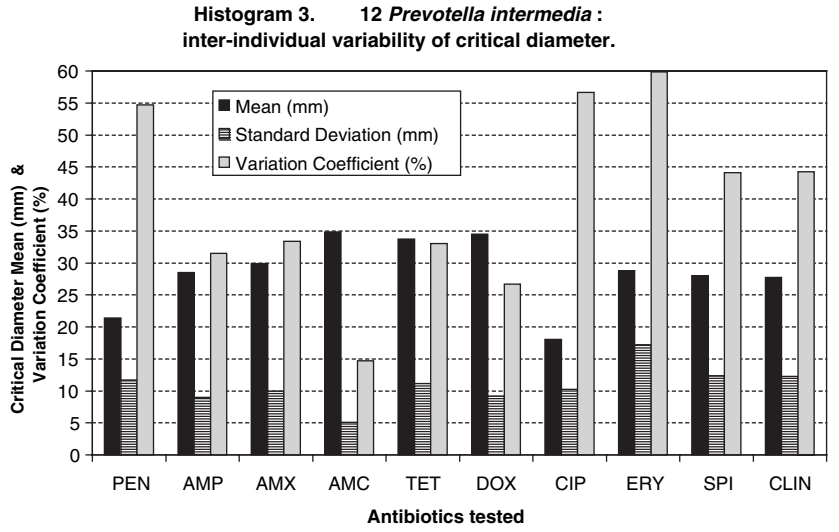

Fig. 3. Histogram of 12 *P. intermedia* bacteria: interindividual variability of critical diameter.

30.80%) and *P. gingivalis* (mean VC: 29.90%) showed intermediate variation.

Antibiotics that showed the highest activity variation against the 50 anaerobic periopathogens were, in decreasing order: ERY (VC: 53.31%); CIP (VC: 52.47%); CLIN (VC: 43.16%); PEN (VC: 41%), and SPI (VC: 40.23%). The most active antimicrobials which showed the lowest variation were: AMC (VC: 14.40%); AMX (VC: 21.13%); AMP (VC: 21.38%), and DOX (VC: 23.05%).

Except for PEN, therefore, aminopenicillins (mean VC: 18.97%) and cyclines (mean VC: 25.75%) demonstrated a very steady activity against anaerobic periopathogens. On the other hand, CIP and macrolides (mean VC: 45.57%) had a very variable activity against them: Pajukanta et al. (45) have previously found major discrepancies in the activity of ciprofloxacin against *P. gingivalis*. Among these antibiotics of high variability, SPI remained the most suitable (in terms of activity and variability).

The finding that 13% of the *P. gingivalis* strains were resistant to both PEN and CLIN may appear surprising. In fact, the strains resistant to PEN were not always the same ones that were resistant to CLIN.

Critical analysis

The anaerobic chamber used in this investigation allowed us to increase the quantity of isolated periopathogens: the sample size remained highly valid for the 50 anaerobic periopathogens on the whole. However, the quantity was certainly small and restricted for the 6 *P. micros* and the 8 *T. forsythia*, which are generally difficult to culture due to their slow growth *in vitro* and fastidious anaerobic nature (21). Further investigations would have to be undertaken with more samples to corroborate our results and to extend them to other periopathogen species: *Capnocytophaga* sp., *Eikenella corrodens,* and especially *Actinobacillus actinomycetemcomitans* (14, 67).

Metronidazole has voluntarily not been included for testing in our study because of its particularly well known narrow spectrum. Lindhe et al. (34) found that this antibiotic had a marked and persistent effect on spirochetes in the subgingival plaque, whereas motile rods were not noticeably affected. These results have some common traits with findings by Listgarten et al. (35). Metronidazole will be the subject of a special experimental investigation in our laboratory.

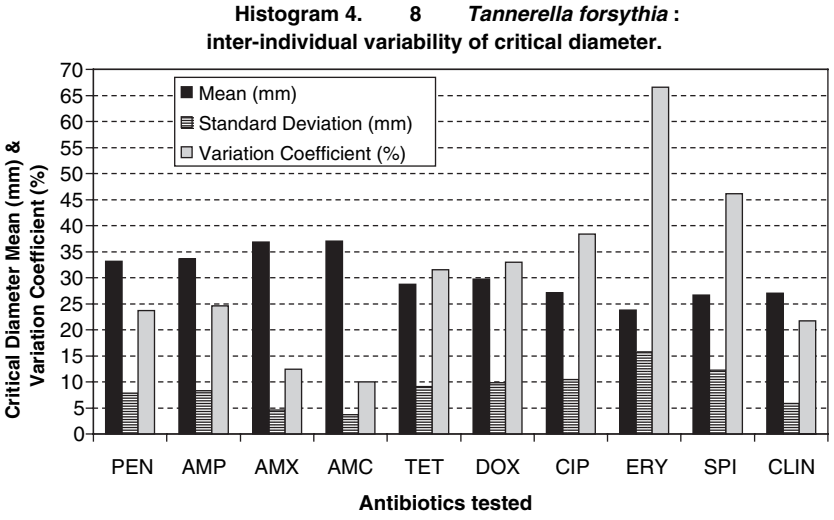

Fig. 4. Histogram of 8 *T. forsythia* bacteria: interindividual variability of critical diameter.

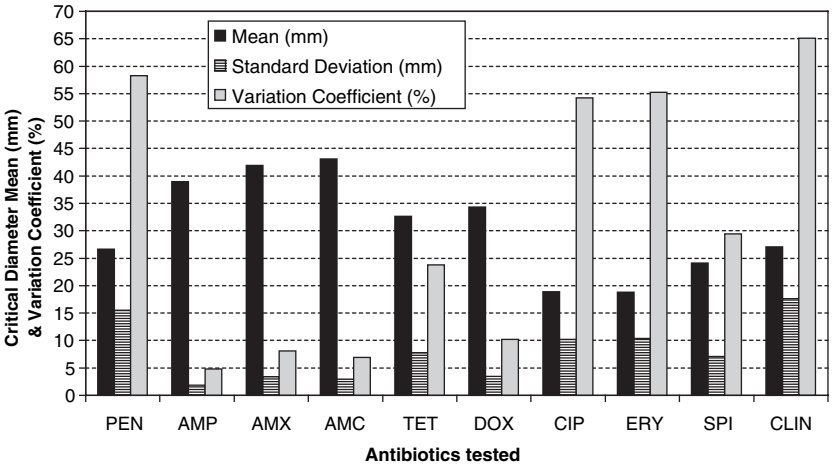

Fig. 5. Histogram of 9 *F. nucleatum* bacteria: interindividual variability of critical diameter.

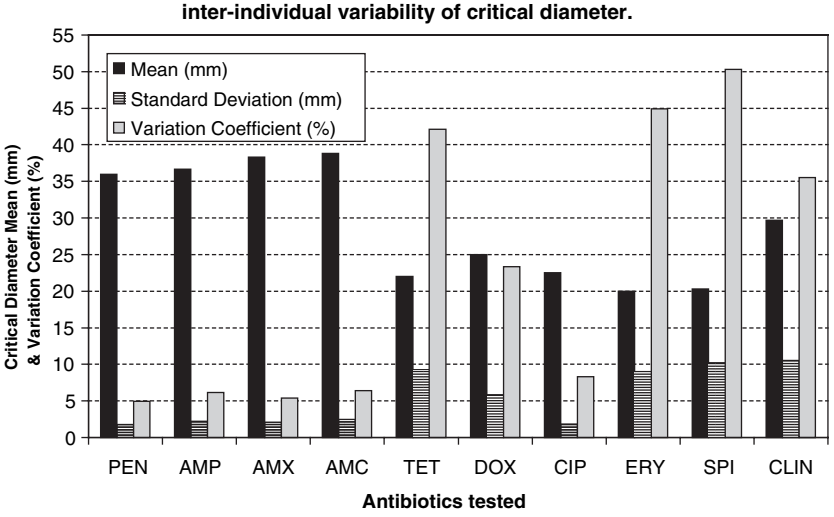

Fig. 6. Histogram of 6 *P. micros* bacteria: interindividual variability of critical diameter.

Clinical consequences

On the basis of the results of our study, it would appear that periodontologists should try to use active antibiotics that possess the weakest variability: AMC, AMX, AMP or even DOX (however, AMC should not be prescribed routinely). Occasionally, an active antibiotic with a high variability may also be beneficial: CLIN, for instance.

In the case of aggressive and refractory periodontitis, the importance of the laboratory microbiological diagnosis becomes crucial for the treatment's success (23, 35, 41, 51, 56, 57). Where *P. intermedia* is identified, it would be desirable to separately sample and seed 3–5 distant colonies of this naturally quite resistant bacterium to achieve numerous antibiograms. Owing to the natural resistance of *P. intermedia* (13, 50), its substantial pathogenicity and its strong intrinsic variability as proved by our investigation (VC mean: 39.89%), care should be taken with this bacterium: antibiotics with high VC (PEN G, ERY, SPI, etc.) must not be used. Wherever *P. intermedia* is detected in high proportions, combination of an antibiotic with a nitroimidazole would be desirable in aggressive periodontitis (56).

Metabolic interactions arise both between different microorganisms in plaque and between the host and plaque microorganisms (9, 24, 28). The enormous complexity of the flora and the metabolic interactions means that the *in vivo* loss of a given bacterial species after antibiotherapy could involve the death of resistant species that were depending on its catabolism products. It is also necessary to take into account the *in vivo* effect of the biofilm, which would probably necessitate considerably increasing the usually prescribed doses (26, 31, 43, 59). Mechanical therapy (scaling and root planing) remains the only way to get rid of biofilm effect: it must then be done concomitantly or prior to antibiotic prescription (3, 75). These are some possible reasons why the *in vivo* results remain uncertain after antibiotherapy, and can even be contradictory to antibiogram *in vitro* results.

Because of these aspects of antimicrobial therapy, and especially when *P. intermedia* is detected in significant quantities, it may therefore be beneficial to use two antibiotics in combination rather than one antimicrobial drug with a narrow spectrum and/or variable activity (42). Amoxicillin/metronidazole remains the most commonly used combination: its success has been widely reported (8, 12, 14, 46, 48, 67, 77). If it is considered that AMC/nitroimidazole

should not be prescribed as a first time therapy (33), then pivampicillin/nitroimidazole, DOX/nitroimidazole, SPI/nitroimidazole or even CLIN/nitroimidazole are all promising combinations for the treatment of aggressive periodontitis.

The variation in susceptibility disclosed by our investigation indicates that for refractory aggressive periodontitis due to *P. intermedia*, antibiotic combinations may be the only radical solution.

Conclusion

Our results show that, using the DDS test, penicillins (especially AMC, AMP, and AMX), cyclines (especially DOX), and CLIN have a very high activity against the 50 anaerobic bacteria studied here. CIP and ERY have the lowest efficacy against these bacteria. SPI has reasonably good activity, but not against *P. micros*. CIP and CLIN show a very variable activity according to anaerobic bacteria species: they are particularly inactive against *P. gingivalis.* CIP, however, appears very efficient against *T. forsythia* and *P. micros*.

In conclusion, the interindividual susceptibility of the principal periodontal pathogens to antibiotics is irregular. This variability seems to be relatively more important with old molecules (PEN, TET, ERY) than with more recent ones (AMC, AMX, AMP, DOX). Among the 50 anaerobic periopathogens tested, *P. intermedia* appeared to be the least susceptible to penicillins and showed the highest coefficient variation. Ciprofloxacin is not suitable for the eradication of anaerobic bacteria in aggressive periodontitis, particularly against *P. gingivalis*, which showed the lowest critical diameter and the highest VC towards this antibiotic. Ciprofloxacin is more appropriate for enteric rod-pseudomonas periodontal infections (56).

Mechanical therapy (scaling and root planing) just prior to systemic prescription of a combination of two antibiotics, may be the best solution to problems of the biofilm effect, the huge flora complexity, and the variability of susceptibility in refractory aggressive periodontitis. This combination of mechanical and chemotherapeutic prescription is in conformity with the recently published studies (3, 56). Further investigations should be undertaken to elucidate the origin of the variability, inter- and intraindividually.

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