Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 569–574 doi: 10.1111/j.1600-051X.2009.01425.x

Journal of Clinical Periodontology

Prevalence of *tetM*, *tetQ*, *nim* and bla_{TEM} genes in the oral cavities of Greek subjects: a pilot study

Ioannis Ioannidis[1], Dimitra Sakellari[1], Argyro Spala[1], Minas Arsenakis[2] and Antonis Konstantinidis[1]

[1]Department of Preventive Dentistry, Periodontology and Implant Biology, Dental School; and [2]Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University, Thessaloniki, Greece

Ioannidis I, Sakellari D, Spala A, Arsenakis M, Konstantinidis A. Prevalence of tetM, tetQ, nim and bla_{TEM} genes in the oral cavities of Greek subjects: a pilot study. J Clin Periodontol 2009; 36: 569–574. doi: 10.1111/j.1600-051X.2009.01425.x.

Abstract

Aim: To investigate the prevalence of *tetM*, *tetQ*, *nim* and bla_{TEM} antimicrobial resistance genes in subgingival and tongue samples of Greek subjects.

Materials and Methods: Fifty-four subjects participated in the present study. Participants each contributed with one pooled subgingival sample from the mesiobuccal surface of the four first molars and one sample from the tongue. Samples were analysed using polymerase chain reaction for *tetM*, *tetQ*, *nim* and bla_{TEM} genes using the primers and conditions described previously. Subjects were stratified according to periodontal status (health, gingivitis or periodontitis). Intake of any antibiotic for medical or dental reasons during the previous 12 months was also recorded (self-reported). Comparisons within and between groups were performed by applying non-parametric tests (z-test with Bonferroni corrections).

Results: A high prevalence of *tetM*, *tetQ* and bla_{TEM} genes was detected in both tongue and subgingival samples (48.1–82.2%). No differences were observed across genes between periodontally healthy, gingivitis or periodontitis cases, and no statistical correlation was observed between the presence of the bla_{TEM} gene and the intake of β-lactams during the last 12 months (Fisher's exact test, $p > 0.05$).

Conclusions: Findings from the present study suggest a high prevalence of *tetM*, *tetQ* and bla_{TEM}, but not *nim* resistance genes in subgingival and tongue samples from Greek subjects.

Key words: antimicrobial resistance genes; β-lactams; Greek; metronidazole; tetracycline

Accepted for publication 5 April 2009

Antimicrobial resistance has become a widespread phenomenon compromising the efficacy of several valuable antimicrobial agents, sometimes with life-threatening implications.

Misuse of several antimicrobials in medical practice, and over-usage for non-medical reasons, such as in agriculture, fish and animal farming as growth promoters, are among the main reasons for the rapid growth in resistance (Martinez 2008). This resistance has been shown to be carried and transferred by genetic material, characterized as resistance genes, which have been fully described and can be identified for many antimicrobial agents (Cockerill 1999).

Although the contribution of dentistry to the increasing levels of antimicrobial resistance is currently unknown, it has been solidly demonstrated both in the medical and in the dental literature that bacterial resistance in a specific country is strongly correlated with the extent of antimicrobial use. In countries with ''loose'' antimicrobial policies, where access to antimicrobials does not require a prescription, bacterial species resistant to antimicrobials can be routinely observed. A trend towards increasing antimicrobial resistance has been shown for *Streptococcus pneumoniae* on comparing Northern with Southern European countries (Bronzwaer et al. 2002), while an important difference in resistance to β-lactams, clindamycin and metronidazole concerning periodontal microflora has been observed between the Netherlands – which follow a stricter policy – and Spain (van Winkelhoff et al. 2000, 2005).

Greece is considered as a country with a lax antimicrobial policy. According to the report of the European Surveillance of Antimicrobial Consumption project concerning outpatient antibiotic

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
This study was supported by the authors' institutions.

use, Greece had the highest community antibiotic use in Europe in 2003 (Ferech et al. 2006). Most antimicrobials can be purchased over the counter and, therefore, it is reasonable to assume that antimicrobial resistance is widespread (Goosens et al. 2005), although no data exist concerning the extent of this phenomenon in the oral cavities of Greek subjects.

Antimicrobials are frequently prescribed for periodontitis patients, usually as adjuncts to conventional mechanical treatment, but the extent of their usage in Greece is currently unknown. In contemporary periodontology, restricted and targeted antimicrobial prescription is strongly encouraged by reports concerning their effects on the clinical parameters of periodontal disease. Current evidence indicates that periodontitis patients – especially those with aggressive disease or a specific bacterial profile – benefit clinically from the administration of antibiotics (Herrera et al. 2002, Haffajee et al. 2003), always in conjunction with mechanical treatment (Herrera et al. 2008). However, an optimal antibiotic regimen appears to require an individual microbiological assessment, something consistent with not being able to follow uniform antibiotic regimens across Europe due to the above-mentioned differences in antimicrobial susceptibility between countries (van Winkelhoff et al. 2005).

The tetracyclines, metronidazole and β-lactams, in addition to being used to treat dental abscesses, are among the most widely used agents for treating periodontal conditions. Mechanisms of bacterial resistance to these antibiotics have been extensively described and attributed to resistance genes. Concerning the tetracyclines, several genetic determinants encoding ribosomal protection, efflux pumps and enzymatic inactivation have been described (Aminov et al. 2001, 2002). The prevalence of two such genes, *tetM* and *tetQ*, in the periodontal environment has been investigated, and the literature indicates that 81–84% of bacteria carrying *tetM* are not periodontal pathogens, while *tetQ* is carried mainly by *Prevotella* and *Bacteroides* spp. (Olsvik et al. 1994, 1995, Lacroix & Walker 1996, Manch-Citron et al. 2000, Chung et al. 2002). Another gene, *tetW*, also encoding ribosomal protection, has also been detected in the oral cavity as present in both pathogenic and non-pathogenic species (Villedieu et al. 2003). The genetic determinants of bacterial resistance to metronidazole have not been extensively investigated in the oral environment. Nitroimidazole resistance genes *nim* (A–D) carrying this property are found in plasmids or the chromosome and their proposed mechanism of action is the encoding of a reductase that cannot convert the pro-drug into active nitroimidazoles, thus preventing the formation of toxic radicals required for antimicrobial activity (Haggoud et al. 1994, Stubbs et al. 2000). In contrast to the limited literature concerning resistance to metronidazole, several reports describe the resistance of oral bacteria to β-lactams. Important sources of β-lactamases in the periodontal environment are *Prevotella* spp., *Fusobacterium nucleatum*, *Eikenella corrodens* and *Tannerella forsythia*, but not *Porphyromonas gingivalis* (van Winkelhoff et al. 1997, Fosse et al. 1999, Herrera et al. 2000) and their presence also appears to correlate with antibiotic consumption (Herrera et al. 2000). All the above-mentioned genetic determinants of bacterial resistance, when present in the oral cavity, might affect the effectiveness of administered antimicrobials and in addition, due to the close bacterial inter-relations in oral biofilms, these determinants can be transferred between different microbial species.

Although extensive outpatient consumption of antimicrobials in Greece leads to expectations of high resistance, no data exist in the literature concerning the extent of this phenomenon or the presence of antibiotic resistance genetic determinants in the oral cavity.

The aim of the present pilot study was therefore to investigate the prevalence of resistance genes *tetM* and *tetQ*, *nim* and bla_{TEM} in the oral cavities of a Greek population.

Materials and Methods

Subject sample

A total of 54 patients from the Clinic of the Department of Periodontology and Implant Biology, Dental School, Aristotle University, Thessaloniki, Greece, constituted the study population.

The criteria for inclusion were as follows: absence of systemic diseases, no history of periodontal treatment during the last 12 months and presence of at least 20 teeth.

Pregnant or lactating women were excluded from the present study. Smoking status (smoker, non-smoker), as reported by patients, was also recorded.

Antibiotic intake during the last 12 months was also recorded as reported by participants. Specifically, subjects filled in a questionnaire concerning any antibiotics administered (trademark) for medical or dental reasons, or used without a prescription. Where possible, answers were cross-checked with the prescriptions.

Subjects were stratified according to their periodontal status, as follows: subjects were considered as periodontally healthy cases when they displayed no probing depth or probing attachment level >3 mm and $<20\%$ of bleeding sites, and as gingivitis cases when they displayed no probing depth or probing attachment level >3 mm and $>20\%$ of bleeding sites, but no radiographic signs of bone loss. Subjects were considered as periodontitis cases (chronic or aggressive) according to the analytical criteria of the American Academy of Periodontology (Armitage 1999).

The study was conducted according to the protocol outlined by the Research Committee, Aristotle University of Thessaloniki, Greece, and was approved by the Ethical Committee of the School of Dentistry.

Clinical sampling and recordings

Two clinical samples were collected from each patient: a pooled subgingival plaque sample from the mesiobuccal surface of the four first molars (or premolars when molars were missing) taken with sterile Gracey curettes, and a sample collected from the dorsal surface of the tongue with a sterile microbiological loop, after applying three consecutive strokes. All samples were immediately placed in 200 μl of TE buffer (Tris HCL 10 mM, EDTA 1 mM, pH 7.5) and stored at -20°C, until assayed.

The following parameters were recorded by the same calibrated examiner (D. S.) at sampling sites, using a manual Williams probe (POW, Hu-Friedy, Chicago, IL, USA).

(a) Probing depth.
(b) Clinical attachment level.
(c) Bleeding on probing.
(d) Presence or absence of plaque (Hygiene Index).

Sampling always preceded clinical recordings.

Polymerase chain reaction (PCR)

First of all, the PCR was performed for the detection of the 16S ribosomal RNA gene, in order to verify that the clinical samples contained quality bacterial DNA (Goncharoff et al. 1993, Manch-Citron et al. 2000). Samples were further analysed by PCR for the presence of genes encoding resistance to tetracycline (*tetQ* and *tetM*), imidazoles (*nim*) and β-lactams (bla_{TEM}). The final volume of the reaction mixture for each PCR assay was 50 μl, containing 5 μl of DNA sample, 200 μM of each deoxynucleotide triphosphate, 5 μl of 1X Standard Taq Reaction Buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3), 0.5 μM of each primer, 2.5 U Taq DNA Polymerase (New England Biolabs Inc., Ipswich, MA, USA) and distilled water. A Peltier Thermal Cycler (PTC-100, Peltier Thermal Cycler, MJ Research, Waltham, MA, USA) was used for PCR.

16S rRNA

The sequences of the *16S rRNA* primers were 5′-CAG GAT TAG ATA CCC TGG TAG TCC ACG C-3′ and 5′-GAC GGG CGG TGT GTA CAA GGC CCG GGA ACG-3′, as described by Goncharoff et al. (1993). PCR parameters included an initial denaturation step at 95°C for 5 min. and 34 cycles of denaturation at 94°C for 1 min., annealing at 55°C for 1 min. and elongation at 72°C for 34 s, followed by a final elongation step at 72°C for 3 min.

tetQ

The sequences of the *tetQ* primers were 5′-GGC TTC TAC GAC ATC TAT TA-3′ and 5′-CAT CAA CAT TTA TCT CTC TG-3′, as described by Lacroix & Walker (1996). PCR parameters included an initial denaturation step at 95°C for 5 min. and 37 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min. and elongation at 72°C for 2 min. and 40 s.

tetM

The sequences of the *tetM* primers were 5′-GAC ACG CCA GGA CAT ATG G-3′ and 5′-TGC TTT CCT CTT GTT CGA G-3′, as described by Lacroix & Walker (1995). PCR parameters included an initial denaturation step at 95°C for 5 min. and 37 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min. and elongation at 72°C for 1 min. and 30 s.

nim

The sequences of the *nim* primers were 5′-ATG TTC AGA GAA ATG CGG CGT AAG CG-3′ and 5′-GCT TCC TTG CCT GTC ATG TGC TC-3′, as described by Trinh & Reysset (1996). PCR parameters included an initial denaturation step at 94°C for 10 min. and 32 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 1 min. and elongation at 72°C for 1 min., followed by a final elongation step at 72°C for 10 min.

bla_{TEM}

The sequences of the bla_{TEM} primers were 5′-ATG AGT ATT CAA CAT TTC CG-3′ and 5′-CCA ATG CTT AAT CAG TGA GG-3′, as described by Arlet et al. (1995). PCR parameters included an initial denaturation step at 94°C for 4 min. and 35 cycles of denaturation at 94°C for 1 min., annealing at 55°C for 1 min. and elongation at 72°C for 1 min., followed by a final elongation step at 72°C for 7 min.

For each set of samples analysed by PCR a negative and a positive control were used. The products of the DNA amplification were electrophoresed through a 1% agarose gel, stained with ethidium bromide, exposed under UV light and photographed. A 100 bp DNA ladder (New England Biolabs Inc.) was also loaded on agarose gel as a molecular weight standard. The amplified fragment sizes were 625 bp for *16S rRNA*, 754 bp for *tetQ*, 397 bp for *tetM*, 458 bp for *nim* and 858 bp for bla_{TEM}.

The electrophoresis for each PCR product was carried out twice in order to test the reproducibility of the method.

Statistical analysis

The statistical analysis of the data was carried out with the statistical packages SPSS (14.0 version) and Instat® (Graphpad Inc., San Diego, CA, USA).

Differences in the prevalence of investigated genes were sought among periodontally healthy, gingivitis and periodontitis subjects, both for tongue and plaque samples, by applying the *z*-test for proportions adjusted with Bonferroni corrections. The same test was applied in order to determine differences within each group (periodontally healthy, gingivitis and periodontitis subjects). The experiment was set to have at least 80% power to detect changes of 25% with a significance level (α) of 0.05 (two tailed).

Correlation between antibiotic intake and the presence of resistance genes both in plaque and in the tongue was sought with Fisher exact test with a significance level of 0.05.

For clinical parameters, indicators of descriptive statistics were used, such as mean and standard deviation for each group, with the patient as the observational unit. Differences in the clinical parameters were sought by applying one-way analysis of variance and the Tukey–Kramer multiple comparisons test, with a significance level of 0.05.

Results

Demographic data for participants and clinical parameters are presented in Table 1. According to the findings of the present study, 51.85% of subjects reported systemic antibiotic intake for various medical reasons during the previous 12 months (Table 1). One subject reported intake of doxycycline, two subjects of metronidazole and the remaining 25 subjects ($\sim$ 50%) reported intake of β-lactamic antibiotics during the previous 12 months. The statistical correlation between antibiotic intake (self-reported) for this specific class of antibiotics was sought, but no correlation was observed between intake of any β-lactam during the previous 12 months and the presence of the bla_{TEM} gene both in subgingival and in tongue samples (Fisher's exact test, $p > 0.05$).

In the present study, *nim* genes were not detected in any subgingival or tongue sample analysed and therefore this gene was excluded from further statistical analysis. In contrast, a high prevalence of *tetM*, *tetQ* and bla_{TEM} resistance genes was observed in both subgingival and tongue samples (Table 2).

When comparing the prevalence of these genes between subjects with different periodontal conditions (Table 2), no differences were observed for the same gene for both subgingival and tongue samples (pairwise comparisons with Bonferroni corrections, $p > 0.05$).

When comparing the prevalence of the investigated genes within the same periodontal status group for both subgingival and tongue samples, no differences were observed within the periodontally healthy, the gingivitis or the periodontitis groups (pairwise com-

Table 1. Demographic data and clinical parameters of subject sample

Diagnosis	Male	Female	Age range	Smokers	Non-smokers	Antibiotic intake during last year	Probing depth (mm) (mean ± SD)	Probing attachment level (mm) (mean ± SD)	Bleeding on probing (mean ± SD)	Hygiene index (mean ± SD)
Periodontal health	6	11	19–48	6	11	8	1.82 ± 0.82 (a)	2 ± 0.82 (a)	0.1 ± 0.3 (a,b)	0.11 ± 0.31 (a)
Gingivitis	4	9	22–61	6	7	5	1.83 ± 0.24 (b)	1.92 ± 0.28 (b)	0.55 ± 0.16 (a)	0.37 ± 0.12 (b)
Chronic periodontitis	9	15	35–76	16	8	15	3.48 ± 0.87 (a,b)	4.35 ± 1.19 (a,b)	0.67 ± 0.35 (b)	0.77 ± 0.41 (a,b)
Total	19	35	19–76	28	26	28				

Values followed by the same letter differ statistically significantly (ANOVA with Tukey–Kramer Multiple Comparisons test, $p < 0.05$).

Table 2. Prevalence of antibiotic resistance genes in subgingival and tongue samples according to periodontal conditions

Diagnosis	*tetQ*		*tetM*		*bla*$_{TEM}$		*nim*	
	plaque % (positive/all)	tongue % (positive/all)	plaque % (positive/all)	tongue % (positive/all)	plaque % (positive/all)	tongue % (positive/all)	plaque	tongue
Health	76.5 (13/17)	85.7 (12/14)	82.4 (14/17)	100 (14/14)	47.1 (8/17)	71.4 (10/14)	ND	ND
Gingivitis	69.2 (9/13)	72.7 (8/11)	76.9 (10/13)	72.7 (8/11)	46.2 (6/13)	72.7 (8/11)	ND	ND
Periodontitis	66.7 (16/24)	75 (15/20)	70.8 (17/24)	75 (15/20)	50 (12/24)	70 (14/20)	ND	ND
Total	70.4 (38/54)	77.8 (35/45)	75.9 (41/54)	82.2 (37/45)	48.1 (26/54)	71.1 (32/45)	ND	ND

ND, not detected.

parisons with Bonferroni corrections, $p > 0.05$).

Discussion

The present pilot study investigated the prevalence of resistance genes *tetM*, *tetQ*, *nim* and *bla*$_{TEM}$ in the oral cavity of a group of Greek subjects.

These genes were selected for several reasons. With regard to the tetracyclines, both *tetM* and *tetQ* are known to provide ribosomal protection, therefore altering the protein target of this class of antimicrobials on the 30S ribosomal subunit of bacteria (Roberts 1996, Aminov et al. 2001, Chopra & Roberts 2001). The high prevalence of these genes – especially *tetM* – observed in the present study is in agreement with previous reports showing their frequent occurrence in many different Gram-positive or Gram-negative bacterial genera in the oral cavity (Olsvik et al. 1994, 1995, Lacroix & Walker 1995, 1996, Villedieu et al. 2003). The source of *tet* genes in the present study, even in subjects who did not report previous exposure to the tetracyclines, is unknown. Greece has complied with European Community legislative references for application of tetracycline, chlortetracycline and oxytetracycline as food additives in poultry (Castanon 2007), but it is known that these substances have been widely used for non-antimicrobial purposes and therefore the presence of tetracycline resistance genes in the environment is to be expected. It has been shown that natural environments represent reservoirs of antibiotic resistance genes and an abundance of *tet* genes has been detected in production animals (Aminov et al. 2001) and water reservoirs near animals farms (Peak et al. 2007). Alarmingly, it has also recently been reported that contamination of rivers by quinolones results in enhanced presence of *qnr* resistance genes in waterborne bacteria (Cattoir et al. 2008). Unfortunately, these data suggest that the periodontal microflora, because of overuse, might have become increasingly resistant to the tetracyclines as already shown by Walker between 1980–1985 and 1991–1995 (Walker 1996).

Bacterial resistance to metronidazole has been less extensively observed. Resistant strains of *Helicobacter pylori* have been described and their presence correlated with the previous administration of metronidazole (Banatvala et al. 1994). In the present study, the sequence common to the four *nim* genes was not detected in any of the subgingival or tongue samples analysed. Carriage of these genes is probably not the only mechanism of resistance to the imidazoles and they have been detected in resistant *Bacteroides*, *Prevotella*, *Porphyromonas* and *Fusobacterium* spp. strains from hospitalized patients in Greece (Katsandri et al. 2006). It has been reported that resistance to metronidazole in Gram-negative anaerobes is considered low in subgingival samples. Listgarten et al. (1993) have reported that 0–2% of these anaerobes from patients with refractory periodontitis are resistant to metronidazole, but it is not known whether this property is due to *nim* genes, and Feres et al. (2002) reported that the 30–50% resistant to metronidazole strains from periodontitis patients are mainly Gram-positive aerobic or facultative species, while major anaerobic periodontal pathogens appear to be susceptible to this antibiotic.

In contrast, a high prevalence of the *bla*$_{TEM}$ gene, thus to the DNA sequence responding to the whole mature TEM β-lactamase, has been observed in the present study (Table 2). TEM β-lactamases are widespread in Gram-negative bacteria and their emerging mutants, characterized as ''extended-spectrum'' β-lactamases or ESBLs, are known to attack several β-lactamic antibiotics (Livermore 1995, Bradford 2001). Although 50% of the subjects reported systemic intake of a β-lactam during the last 12 months, no statistical correlation was observed between the presence of the *bla*$_{TEM}$ gene and the systemic intake of these antibiotics (Fisher's exact test, $p > 0.05$).

The approach used in the present study did not identify the bacterial source of these resistance genes. It has been shown previously that high rates of conjugation frequently exist within biofilms and in model dental plaque (Hausner & Wuertz 1999, Roberts et al. 1999,

2001). In addition, this phenomenon was recently observed in vivo, when a transposon conferring resistance to doxycycline was transferred between two streptococci species in a patient undergoing antibiotic treatment (Warburton et al. 2007). Therefore, when present, as observed in the present study, these resistance genes might be transferred from non-pathogenic to pathogenic species in the complex oral ecosystem and further disseminate resistance.

Although the role of dentistry in the development of resistance is largely unclear (Haas et al. 1998), data from the present pilot study are indicative of the extent of resistance in the oral cavity in a country with widespread outpatient antibiotic consumption. These genetic determinants are essential elements of this property and their presence might compromise the clinical effectiveness of popular classes of antibiotics for treating oral infections. Therefore, dentists should be aware of this phenomenon and follow appropriate prescription policies as suggested by the medical community (Scheld 2003). These policies revolve around the objectives of only prescribing antimicrobial therapy when it is beneficial and using agents with optimal activity against suspected pathogens. In periodontology, several authors have extensively reported and described the value of bacterial testing before adjunctive antibiotic treatment to select the most specific antibiotics (Mombelli 2005, van Winkelhoff 2005, van Winkelhoff & Winkel 2005, Haffajee 2006). The strategy for selection of specific antibiotics in the treatment of periodontal disease should also include, in addition to identification of periodontal pathogens, knowledge of the pharmacological characteristics of various agents that affect their concentration in gingival crevicular fluid (AAP 2004). The combination of this knowledge with the relation of achievable antibiotic concentrations in GCF to known minimum inhibitory concentrations for various pathogens has been shown to be a more efficient strategy for selecting antibiotics, under the constraints of the biofilm structure (Beikler et al. 2004).

The findings from the present study, which indicate a high prevalence of resistance genes in the oral cavity in a country with extensive antibiotic consumption, also suggest that bacterial testing and guided antimicrobial prescription for periodontal patients, in addition to reducing the risk of the development and dissemination of antibiotic resistance, might also enhance clinical benefits for periodontal patients. In addition to the above-mentioned parameters, which should be taken into consideration when prescribing systemic antibiotics in periodontology, information concerning the presence of genetic determinants of bacterial resistance might also assist in selecting an effective and specific antimicrobial agent.

In conclusion, data from the present study suggest a high prevalence of *tetM*, *tetQ* and bla_{TEM}, but not *nim* genes in the oral cavity of Greek subjects.

Acknowledgements

The authors would like to thank Mrs. Theodora Slini, MSc, for her contribution to statistical analysis and Dr. Joseph Papaparaskevas, Lecturer, Department of Microbiology, Medical School, National and Kapodistrian University of Athens, Greece, for kindly providing positive control for *nim* genes.

References

AAP. (2004) Systemic antibiotics in periodontics. *Journal of Periodontology* **75**, 1553–1565.

Aminov, R. I., Chee-Sanford, J. C., Garrigues, N., Teferedegne, B., Krapac, I. J., White, B. A. & Mackie, R. I. (2002) Development, validation and application of PCR primers for detection of tetracycline efflux genes of Gram-negative bacteria. *Applied and Environmental Microbiology* **68**, 1786–1793.

Aminov, R. I., Garrigues-Jeanjean, N. & Mackie, R. I. (2001) Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Applied and Environmental Microbiology* **67**, 22–32.

Arlet, G., Brami, G., Decre, D., Flippo, A., Gaillot, O., Lagrange, P. H. & Philippon, A. (1995) Molecular characterisation by PCR-restriction fragment length polymorphism of TEM β-lactamases. *FEMS Microbiology Letters* **134**, 203–208.

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Banatvala, N., Davies, G. R., Abdi, Y., Clements, L., Rampton, D. S., Hardie, J. M. & Feldman, R. A. (1994) High prevalence of *Helicobacter pylori* metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastroduodenal disease. *Gut* **35**, 1562–1566.

Beikler, T., Prior, K., Ehmke, B. & Flemmig, T. F. (2004) Specific antibiotics in the treatment of periodontitis – a proposed strategy. *Journal of Periodontology* **75**, 169–175.

Bradford, P. A. (2001) Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews* **14**, 933–951.

Bronzwaer, S. L. A. M., Cars, O., Buchholz, U., Mölstad, S., Goettsch, W., Veldhuijzen, I. K., Kool, J. L., Sprenger, M. J. W., Degener, J. E. & participants in the European Antimicrobial Resistance Surveillance System. (2002) A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerging Infectious Diseases* **8**, 278–282.

Castanon, J. I. R. (2007) History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science* **86**, 2466–2471.

Cattoir, V., Poirel, L., Aubert, C., Soussy, C. J. & Nordmann, P. (2008) Unexpected occurrence of plasmid-mediated Quinolone resistance determinants in environmental *Aeromonas* spp. *Emerging Infectious Diseases* **14**, 231–237.

Chopra, I. & Roberts, M. (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews* **65**, 232–260.

Chung, W. O., Gabany, J., Perrson, G. R. & Roberts, M. C. (2002) Distribution of erm(F) and tet(Q) genes in four oral bacterial species and genotypic variation between resistant and susceptible isolates. *Journal of Clinical Periodontology* **29**, 152–158.

Cockerill, F. R. (1999) Genetic methods for assessing antimicrobial resistance. *Antimicrobial Agents and Chemotherapy* **43**, 199–212.

Ferech, M., Coenen, S., Malhotra-Kumar, S., Dvorakova, K., Hendrickx, E., Suetens, C. & Goosens, H. (2006) European surveillance of antimicrobial consumption (ESAC): outpatient antibiotic use in Europe. *Journal of Antimicrobial Chemotherapy* **58**, 401–407.

Feres, M., Haffajee, A. D., Allard, K., Som, S., Goodson, J. M. & Socransky, S. S. (2002) Antibiotic resistance of subgingival species during and after antibiotic therapy. *Journal of Clinical Periodontology* **29**, 724–735.

Fosse, T., Madinier, I., Hitzig, C. & Charbit, Y. (1999) Prevalence of beta-lactamase-producing strains among 149 anaerobic gram-negative rods isolated from periodontal pockets. *Oral Microbiology and Immunology* **14**, 352–357.

Goncharoff, P., Figurski, D. H., Stevens, R. H. & Fine, D. H. (1993) Identification of *Actinobacillus actinomycetemcomitans*: polymerase chain reaction of *lktA*-specific sequences. *Oral Microbiology and Immunology* **8**, 105–110.

Goosens, H., Ferech, M., Vander Stichele, R. & Elseviers, M. (2005) Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* **365**, 579–587.

Haas, D. A., Epstein, J. B. & Eggert, F. M. (1998) Antimicrobial resistance: dentistry's role. *Journal of the Canadian Dental Association* **64**, 496–502.

Haffajee, A. D. (2006) Systemic antibiotics: to use or not to use in the treatment of periodontal infections. That is the question. *Journal of Clinical Periodontology* **33**, 359–361.

Haffajee, A. D., Socransky, S. S. & Gunsolley, J. C. (2003) Systemic anti-infective periodontal therapy. A systematic review. *Annals of Periodontology* **8**, 115–181.

Haggoud, A., Reysset, G., Azeddoug, H. & Sebald, M. (1994) Nucleotide sequence analysis of two 5-nitroimidazole resistance determinants from Bacteroides strains and of a new insertion sequence upstream of the two genes. *Antimicrobial Agents and Chemotherapy* **38**, 1047–1051.

Hausner, M. & Wuertz, S. (1999) High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Applied and Environmental Microbiology* **65**, 3710–3713.

Herrera, D., Alonso, B., León, R., Roldán, S. & Sanz, M. (2008) Antimicrobial therapy in periodontitis: the use of systemic antimicrobials against the subgingival biofilm. *Journal of Clinical Periodontology* **35** (Suppl. 8), 45–66.

Herrera, D., Sanz, M., Jepsen, S., Needleman, I. & Roldán, S. (2002) A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. *Journal of Clinical Periodontology* **29** (Suppl. 3), 136–159.

Herrera, D., van Winkelhoff, A. J., Dellemijn, K., Winkel, E. G. & Sanz, M. (2000) Beta-lactamase producing bacteria in the subgingival microflora of adult patients with periodontitis: a comparison between Spain and the Netherlands. *Journal of Clinical Periodontology* **27**, 520–525.

Katsandri, A., Avlamis, A., Pantazatou, A., Houhoula, D. P. & Papaparaskevas, J. (2006) Dissemination of *nim*-class genes, encoding nitroimidazole resistance, among different species of Gram-negative anaerobic bacteria isolated in Athens, Greece. *Journal of Antimicrobial Chemotherapy* **58**, 705–706.

Lacroix, J. M. & Walker, C. B. (1995) Detection and incidence of the tetracycline resistance determinant *tet(M)* in the microflora associated with adult periodontitis. *Journal of Periodontology* **66**, 102–108.

Lacroix, J. M. & Walker, C. B. (1996) Detection and prevalence of the tetracycline resistance determinant *TetQ* in the microbiota associated with adult periodontitis. *Oral Microbiology and Immunology* **11**, 282–288.

Listgarten, M. A., Lai, C. H. & Young, V. (1993) Microbial composition and pattern of antibiotic resistance in subgingival microbial samples from patients with refractory periodontitis. *Journal of Periodontology* **64**, 155–161.

Livermore, D. M. (1995) Beta-Lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews* **8**, 557–584.

Manch-Citron, J. N., Lopez, G. H., Dey, A., Rapley, J. W., MacNeill, S. R. & Cobb, C. M. (2000) PCR monitoring for tetracycline resistance genes in subgingival plaque following site-specific periodontal therapy. A preliminary report. *Journal of Clinical Periodontology* **27**, 437–446.

Martinez, J. L. (2008) Antibiotics and antibiotic resistance genes in natural environments. *Science* **321**, 365–367.

Mombelli, A. (2005) Antimicrobial profiles of periodontal pathogens and systemic antimicrobial therapy. *Journal of Clinical Periodontology* **32**, 891–892.

Olsvik, B., Hansen, B. F., Tenover, F. C. & Olsen, I. (1995) Tetracycline-resistant microorganisms recovered from patients with refractory periodontal disease. *Journal of Clinical Periodontology* **22**, 391–396.

Olsvik, B., Olsen, I. & Tenover, F. C. (1994) The tet(Q) gene in bacteria isolated from patients with refractory periodontal disease. *Oral Microbiology and Immunology* **9**, 251–255.

Peak, N., Knapp, C. W., Yang, R. K., Hanfelt, M. M., Smith, M. S., Aga, D. S. & Graham, D. W. (2007) Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environmental Microbiology* **9**, 143–151.

Roberts, A. P., Cheah, G., Ready, D., Pratten, J., Wilson, M. & Mullany, P. (2001) Transfer of TN916-like elements in microcosm dental plaques. *Antimicrobial Agents and Chemotherapy* **45**, 2943–2946.

Roberts, A. P., Pratten, J., Wilson, M. & Mullany, P. (1999) Transfer of a conjugative transposon, Tn5397 in a model oral biofilm. *FEMS Microbiology Letters* **177**, 63–66.

Roberts, M. C. (1996) Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility and distribution. *FEMS Microbiology Reviews* **19**, 1–24.

Scheld, W. M. (2003) Maintaining fluoroquinolone class efficacy: review of influencing factors. *Emerging Infectious Diseases* **9**, 1–9.

Stubbs, S. L. J., Brazier, J. S., Talbot, P. R. & Duerden, B. I. (2000) PCR-restriction fragment length polymorphism analysis for identification of *Bacteroides* spp. and characterization of nitroimidazole resistance genes. *Journal of Clinical Microbiology* **38**, 3209–3213.

Trinh, S. & Reysset, G. (1996) Detection by PCR of the nim genes encoding 5-nitroimidazole resistance in Bacteroides spp. *Journal of Clinical Microbiology* **34**, 2078–2084.

van Winkelhoff, A. J. (2005) Antibiotics in periodontics: are we getting somewhere? *Journal of Clinical Periodontology* **32**, 1094–1095.

van Winkelhoff, A. J., Herrera, D. II, Oteo, A. & Sanz, M. (2005) Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in the Netherlands and Spain. *Journal of Clinical Periodontology* **32**, 893–898.

van Winkelhoff, A. J., Herrera, G., Winkel, E. G., Dellemijn, K., Vandenbroucke, G. & Sanz, M. (2000) Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis: a comparison between the Netherlands and Spain. *Journal of Clinical Periodontology* **27**, 79–86.

van Winkelhoff, A. J. & Winkel, E. G. (2005) Microbiological diagnostics in periodontics: biological significance and clinical validity. *Periodontology 2000* **39**, 40–52.

van Winkelhoff, A. J., Winkel, E. G., Barendregt, D., Dellemijn, K., Stijne, A. & van der Velden, U. (1997) Beta-Lactamase producing bacteria in adult periodontitis. *Journal of Clinical Periodontology* **24**, 538–543.

Villedieu, A., Diaz-Torres, M. L., Hunt, N., McNab, R., Spratt, D. A., Wilson, M. & Mullany, P. (2003) Prevalence of tetracycline resistance genes in oral bacteria. *Antimicrobial Agents and Chemotherapy* **47**, 878–882.

Walker, C. B. (1996) The acquisition of antibiotic resistance in the periodontal microflora. *Periodontology 2000* **10**, 79–88.

Warburton, P. J., Palmer, R. M., Munson, M. A. & Wade, W. G. (2007) Demonstration of *in vivo* transfer of doxycycline resistance mediated by a novel transposon. *Journal of Antimicrobial Chemotherapy* **60**, 973–980.

Address:
Dimitra Sakellari
88 Mitropoleos street
Thessaloniki 54622
Greece
E-mail: dimisak@med.auth.gr

Clinical Relevance

Scientific rationale for study: Resistance to antimicrobials is a widespread phenomenon, influenced by the antimicrobial policy of each country. Data concerning the prevalence of resistance genes could affect the strictness of the prescription policy of dental professionals as well as assist them in educating the public in prudent use of antimicrobials.
Principal findings: A high prevalence of resistance genes to the tetracyclines and to β-lactamic antibiotics, but not to metronidazole, was observed in both subgingival and tongue samples from Greek subjects.
Practical implications: The high prevalence of the resistance genes investigated might affect the efficacy of these classes of antibiotics for treating oral infections.