Distribution of *Aggregatibacter actinomycetemcomitans* in deep caries lesions

Irena Kuzmanović Radman¹, Aleksandra Đeri¹, Adriana Arbutina², Jelena Milašin³, Ljiljana Sabljić Amidižić⁴

¹Department of Dental Diseases, Faculty of Medicine, Study program of Dentistry, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; ²Department of Orthodontics, Faculty of Medicine, Study program of Dentistry, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; ³University of Belgrade, Faculty of Dentistry, Belgrade, Serbia; ⁴Institute for Clinical Pathology UCCRS

**SUMMARY**

**Introduction** Deep caries is a reversible process where caries lesion has affected bigger part of dentin and only thin layer of softened dentin that separates lesion from the pulp is remained. The objective of this study was to identify and determine serotypes of *Aggregatibacter actinomycetemcomitans* in teeth with deep caries lesions at the beginning of their treatment.

**Material and methods** Clinical research included 29 patients of both genders, aged 16 to 40 and 45 permanent teeth with diagnosed deep caries lesions based on medical history, clinical and radiographic examination. After cavity preparation and removal of softened dentin, microbiological swab was taken from the bottom of the cavity. Swabs were disposed in special sterile micro tubes and stored at the temperature of -80°C until serotyping was done (determination of serotypes of *A. actinomycetemcomitans* bacterium).

**Results** In one of the 3 samples two serotypes of *A. actinomycetemcomitans* (b and c) were identified which is relatively rare finding, while in the second and third sample serotypes (a) and serotype (b) was identified, respectively.

**Conclusion** In the three samples the 3 serotypes were found (a, b and c) and one of the samples was carrying even two different serotypes, which is a rare phenomenon. For more serious epidemiological study of *A. Actinomycetemcomitans* serotypes at the population level incomparably larger starting material is necessary, at least few hundred of samples.

**Keywords:** deep caries lesions; *Aggregatibacter actinomycetemcomitans*; serotypes; PCR

**INTRODUCTION**

Dental caries is a chronic complex bacterial infection that results in milligram loss of minerals from infected tooth. Some authors have defined caries as a disease of hard dental tissues (enamel, dentin and cementum) with characteristic processes of demineralization and remineralisation. Deep caries can be classified as clinically visible lesion in dentin characterized by close topographic relation to the pulp, followed by weakening of the sidewalls due to the progression of caries both in width and depth [1, 2].

One of the factors affecting the occurrence of caries is dental plaque that represents adhered deposits of bacteria and their products and it exists on every surface of teeth. Dental plaque contains pyogenic bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* [3]. According to the new classification based on phylogenetic similarity, *A. actinomycetemcomitans* was in 2006 grouped together with *Haemophilus arophilus* and *H. paraphrophilus* in the new Aggregatibacter genus. *A. actinomycetemcomitans* was the first time described in 1912 by Klinger as cocobacillary bacteria isolated together with *Actinomyces* from actinomycotic lesions of man and it was therefore originally grouped into the *Actinomyces* genus.

*A. actinomycetemcomitans* is a non-motile, slow-growing, capnophilic Gram-negative cocccobaciul. It grows slowly at 37°C, both aerobically and anaerobically. Five stereotypic groups of *A. actinomycetemcomitans* were classified based on surface polysaccharide, where serotypes a, b and c are the most prevalent in the oral cavity. A particular clone of serotype b shows enhanced leukotoxic activity. It is predominantly associated with the cases of localized aggressive periodontitis, while the serotype c is usually found in healthy subjects [4–7].

Takahashi et al. demonstrated in an animal model that SPA serotype b has an accentuated ability to stimulate interleukin-1 release from macrophages. The latest data based on molecular genetic analysis indicated significant divergent evolutions of genomes of bacteria serotype a in relation to serotypes b/c, and differences in genomes implied accentuated phenotypic differences [8]. Pajukanta et al. showed that the response of *A. Actinomycetemcomitans* to antimicrobials can vary depending on the serotype [9].

**Address for correspondence:** IRENA KUZMANOVIĆ RADMAN, Vojvode Petra Bojovića 1a Street, 78000 Banja Luka, Republika Srpska, Bosnia and Herzegovina; irena.radman78@gmail.com
It is assumed that patients are usually infected with one serotype that is usually maintained over time, i.e. indicates stability [10]. However, more recent research based on molecular genetic testing and not on serological tests, indicated that serotype changes are possible over time. In the study of van der Reijden et al. performed on the population of Indonesian island Java, after 8 years (time interval of the study) there was a change noticed in the prevalence of serotypes at the population level [11]. Among the people who were tested at the beginning and at the end of the study, after 8 years 58% of them had the same bacterial serotypes, and 42% of them had other serotypes. They also reported rare cases of multiple serotype infections, around 10% while during the 8 years of research multiple infections increased from 12% to 17% [12].

Another characteristic of Aggregatibacter actinomycetemcomitans is that distribution of serotypes significantly fluctuates depending on geographic region of analysed population as well as periodontal status of teeth. Thus, for example, in the USA, in patients with localized juvenile periodontitis the serotype b is more common than serotypes a and c. The situation is similar in Finland population where serotype b dominates among the patients with periodontal disease, whereas serotype c is more commonly found among patients with no periodontal disease. In Japanese population, serotypes a, c and e were the most common [13].

The aim of this study was to determine and identify serotypes of Aggregatibacter actinomycetemcomitans in teeth with deep caries lesions at the beginning of deep caries treatment.

**MATERIAL AND METHODS**

The clinical study was conducted on 45 permanent teeth of patients, aged 16 to 40. Different morphology groups of permanent teeth with deep caries lesions were included in the study. Deep lesions considered dental caries followed by sensitivity to thermal stimuli, affecting more than ¾ of the tooth crowns with lots of softened dentin. After cavity preparations and removal of softened dentin, the swab was taken from the bottom of the cavity. Taken swabs were disposed in special sterile micro tubes and stored at the temperature of -80°C until serotyping was performed (determination of serotypes of Aggregatibacter actinomycetemcomitans).

The samples were tested at the Institute for Human Genetics, Faculty of Dentistry, University of Belgrade using the multiplex PCR method that enables simultaneous amplification of various gene sequences using multiple pairs of primers. Familiar sequences of primers were used for PCR reactions. Serotyping of Aggregatibacter actinomycetemcomi-

---

**RESULTS**

Oligonucleotide primers specific for the group of genes involved in the biosynthesis of bacterial serotype-specific polysaccharide antigens were designed to be able to identify five main serotypes of Aggregatibacter actinomycetemcomitans (a, b, c, d and e) by using the multiplex PCR. In laboratory conditions, multiplex PCR optimization has proven to be technically demanding and that is why it was possible to serotypically define only a small percentage of samples. A serotype was conditionally established after a number of repeated attempts in only 3 samples. The interesting fact is that two serotypes (b and c) were found in one of the 3 samples, which is relatively rare finding. Figure 1 shows that gel was given after one attempt of serotyping where only one out of 10 samples showed the corresponding strips (10b sample). In the samples # 18 and # 23 arrows represent strips that do not correspond to any known serotype and which could be the PCR artefacts.

One of repeated serotyping successfully identified serotypes in 2 more samples: 7c (serotype a) and 6 (serotype c). During repeated multiplex PCR reaction, a nonspecific strip that was present in the first experiment was now lost in the sample 23 (Figure 2).

**DISCUSSION**

*Aggregatibacter actinomycetemcomitans*, ie its serotype c, is a part of normal flora of the oral cavity in healthy patients. This bacterium can also be pathogen because it has significant virulence factors (one of them is adhesion) that enable colonisation of bacteria and intensify its destructive potential in oral diseases [14]. Serotyping of bacteria is a suitable typization method for epidemiological studies. Primarily it is easy to perform and more sensitive compared to other methods that require additional equipment and are more costly [15].
Dental caries is a multibacterial disease but serotyping is the only method to determine certain serotypes of bacteria that play role in aetiology. Number of studies has shown that certain bacteria detected in dental plaque are closely associated with the occurrence of caries while large caries lesions often communicate with subgingival biofilm bacteria. *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* are oral pathogenic bacteria associated with caries and periodontal disease. Pspoter et al. (2011) aimed to determine colonization of these two microorganisms in dental plaque of adolescents and 72% of subjects with acute periodontal disease [24]. Tinoco et al. also found *A. actinomycetemcomitans* in 41.6% of the subjects with chronic periodontal disease [23]. The study of Cortelli et al. (2005) showed the presence of *A. actinomycetemcomitans* in 41.6% of the subjects with chronic periodontal disease and 72% of subjects with acute periodontal disease [24]. The literature indicates that it is impossible to determine the serotype in 3 to 8% of samples of *A. Actinomycetemcomitans* [22]. Unfortunately, this percentage was significantly higher in our study. We tried to overcome technical problems in many ways by changing the number of experimental parameters but we got unsatisfactory results. Multiplex PCR was performed with different amounts of starting material and different duration of particular steps of reaction. Also, the number of cycles was modified (25, 30, 35) as well as MgCl2 concentrations and hybridization temperature. However, even after all the effort and repeated attempts the success of serotyping was moderate. We were able to determine *A. Actinomycetemcomitans* serotypes in only 21% of the samples.

Numerous studies have come to conclusion that *A. actinomycetemcomitans* is most frequently associated with periodontal diseases [23]. The study of Cortelli et al. (2005) showed the presence of *A. actinomycetemcomitans* in 41.6% of the subjects with chronic periodontal disease and 72% of subjects with acute periodontal disease [24]. Tinoco et al. also found *A. actinomycetemcomitans* in 80% of young patients with periodontal disease and suggested that the presence of this bacterium in the oral cavity may serve as an indicator of risk for future tests of acute periodontal disease [25].

Based on research of deep caries lesions Simon-Soro and Mira found diverse ecosystem made of a large number of bacteria affecting the spreading of caries lesions. The results showed that *S. mutans* was present in a small percentage and that other bacteria including *A. actinomycetemcomitans* affect spreading of caries lesions [26].

**CONCLUSION**

Given the small initial number of teeth, relatively small percentage of samples positive for *A. actinomycetemcomitans* and finally, poor achievement of the multiplex reaction of serotyping, it is impossible to talk about the prevalence of certain serotypes in our population. In the 3 samples three serotypes of *A. actinomycetemcomitans* (a, b and c) were identified and two different serotypes were
identified in one of the samples that is a rare phenomenon. For more serious epidemiological study of serotypes of *A. Actinomycetemcomitans* at the population level and their relation to the formation of dental caries an incomparably larger starting material is necessary, at least a few hundred of samples.

**REFERENCES**

1. Al-Hiyasat AS, Barrieshi-Nusair KM, Al-Omari MA. The radiographic outcomes of direct pulp-capping procedures performed by dental students: a retrospective study. J Am Dent Assoc. 2006; 137(12):1699–705. [DOI: 10.14219/jada.archive.2006.0116] [PMID: 17138715]

2. Ahmad S, Al-Hiyasat, Kefah M, Barrieshi-Nusair. The radiographic outcomes of direct pulp-capping procedures performed by dental students: a retrospective study. J Am Dent Assoc. 2006; 137(12):1699–705. [DOI: 10.14219/jada.archive.2006.0116] [PMID: 17138715]

3. Arora A, Scott JA, Bhole S, Do L, Schwarz E, Blinkhorn AS. Early childhood feeding practices and dental caries in preschool children: a multi-centre birth cohort study. BMC Public Health. 2011; 11:28. [DOI: 10.1186/1471-2458-11-28] [PMID: 21223601]

4. Norskov-Lauritsen N, Kilian M. Reclassification of *Actinobacillus actinomycetemcomitans, Haemophilus aphrophilus*, *Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include *V* factor-dependent and *V* factor-independent isolates. Int J Syst Evol Microbiol. 2006; 56(9):2135–46. [DOI: 10.1099/ijs.0.64207-0] [PMID: 16957111]

5. Taylor LS, Selwyn DRL. *Aggregatibacter actinomycetemcomitans* (Actinobacillus actinomycetemcomitans). Available from: www.antimicrobe.org/new/b72.asp

6. Zambon JJ, Slots J, Cenco RJ. Serology of oral *Actinobacillus actinomycetemcomitans* and *serotype distribution in human periodontal disease*. Infect Immun. 1983; 41(1):19–27. [PMID: 6407997]

7. Haubek D, Johansson A. Pathogenicity of the highly leukotoxic *JP2* clone of *Aggregatibacter actinomycetemcomitans* and its geographic dissemination and role in aggressive periodontitis. J Oral Microbiol. Published online 2014. [DOI: 10.1097/jom.v6.23980] [PMID: 25206940]

8. Takahashi T, Nishihara T, Ishihara Y, Amano K, Shibuya N, Moro I, et al. Murine macrophage interleukin-1 release by capsularlye specific poly saccharide antigens of *Actinobacillus actinomycetemcomitans*. Infect Immun. 1991; 59(1):18–23. [PMID: 19870352]

9. Pajukanta R, Asikainen S, Saarela M, Alaluusua S, Jousimies-Somer H. In vitro antimicrobial susceptibility of different serotypes of *Actinobacillus actinomycetemcomitans*. Scand J Dent Res. 1993; 101:299–303. [PMID: 1329617]

10. Asikainen S, Lai CH, Alaluusua S, Slots J. Distribution of *Actinobacillus actinomycetemcomitans* serotypes in periodontal health and disease. Oral Microbiol Immunol. 1991; 6(2):115–8. [DOI: 10.1111/j.1399-302X.1991.tb00462.x] [PMID: 1945486]

11. Van der Reijden WA, Bosch-Tijhof CJ, van der Velden U, van Winkelhoff AJ. Java project on periodontal diseases serotype distribution of *Aggregatibacter actinomycetemcomitans* and serotype dynamics over an 8-year period. J Clin Periodontol. 2008; 35(6):487–92. [DOI: 10.1111/j.1600-051X.2008.01218.x] [PMID: 18422698]

12. Suzuki N, Nakano Y, Yoshida Y, Ikeda D, Koga T. Identification of *Actinobacillus actinomycetemcomitans* serotypes by Multiplex PCR. J Clin Microbiol. 2001; 39(5):2002–5. [DOI: 10.1128/JCM.39.5.2002-2005.2001] [PMID: 11326035]

13. Koudouhi B, Zimantart T, Mahdouani K, Hentati H, Bakhrouf A. Antibiotic resistance and adhesion properties of oral *Enterococcus* associated to dental caries. BMC Microbiology. 2011; 29:11:155. [DOI: 10.1186/1471-2180-11-155] [PMID: 21719420]

14. Musiè L, Puhar I. *Aggregatibacter actinomycetemcomitans* - osobna iskaznica parodontopatogene. Sonda. 2011, 15(28):45-8.

15. Stanković Nedeljković N, Kocic B, Tiodorovic B, Branković S, Mladenović Antić S. Serotipizacija i analiza vrsta proizvedenih pigmenata kliničkih izolata *Pseudomonas aerogena*. Vojnosanitetski pregled. 2011; 68(11):923–9. [DOI: 10.2298/VS1111923]

16. Loeches WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev. 1986; 50:533–80. [PMID: 3540569]

17. Van Houze J. Role of microorganisms in caries etiology. J Dent Res. 1994; 73:672–81. [PMID: 8163737]

18. Psoter WJ, Ge Y, Russell SL, Chen Z, Katz RV, Jean-Charles G, et al. PCR detection of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* in dental plaque samples from Haitian adolescents. Clin Oral Investig. 2011; 15(4):461–9. [DOI: 10.1007/s00784-010-0412-6] [PMID: 20454610]

19. Henderson B, Nair SP, Ward JM, Wilson M. Molecular pathogenicity of the oral opportunistic pathogen *Actinobacillus actinomycetemcomitans*. Annu Rev Microbiol. 2003; 57:29–55. [DOI: 10.1146/annurev.micro.57.093002.090908] [PMID: 14527274]

20. Fine DH, Markowitz K, Furgang D, Farlie K, Ferranz J, Nasri C, et al. *Aggregatibacter actinomycetemcomitans* and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. J Clin Microbiol. 2007; 45:3859–69. [DOI: 10.1128/JCM.00653-07] [PMID: 17942668]

21. Sofrata AH, Claesson RL, Lingstrom PK, Gustafsson AK. Strong antibacterial effect of Miswak against oral microorganisms associated with periodontitis and caries. J Periodontol. 2008; 79(8):1474–9. [DOI: 10.1902/jop.2008.070506] [PMID: 18672998]

22. Rylev M, Kilian M. Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol. 2008; 35:346–61. [DOI: 10.1111/j.1600-051X.2008.01280.x] [PMID: 18724862]

23. Kittichotirat W, Burmgarner R, Chen C. Markedly different genome arrangements between serotype a strains and serotypes b or c strains of *Aggregatibacter actinomycetemcomitans*. BMC Genomics. 2010; 11:60-8. [DOI: 10.1186/1471-2164-11-60] [PMID: 19980232]

24. Tinoco EM, Beldi ML, Loureiro CA, Lana M, Campedelli F, Tinoco NM, et al. Localization of *Aggregatibacter actinomycetemcomitans* in a Brazilian population. Eur J Oral Sci. 1997; 105(1):19–14. [DOI: 10.1034/j.1600-051X.1997.t01-10.0413-y] [PMID: 9085023]

25. Cortelli R, Cortelli SC, Jordan S, Haraszy VT, Zambon J. Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol. 2005; 32(8):860–6. [DOI: 10.1111/j.1600-0517-2005.00036.x] [PMID: 15992689]

26. Simon-Soro A, Mira A. Solving the ecology of dental caries. Trends Microbiol. 2015; 23(2):76–82. [DOI: 10.1016/j.tim.2014.10.010] [PMID: 25435135]

Received: 12.07.2016 - Accepted: 11.10.2016
Zastupljenost bakterije Aggregatibacter actinomyctesiumcomitans u dubokim karijesnim lezijama

Irena Kuzmanović Radman1, Aleksandra Đeri1, Adriana Arbutina3, Jelena Milašin3, Ljiljana Sabljić Amidžić4

1Katedra za bolesti zuba, Medicinski fakultet, Studijski program Stomatologija, Univerzitet u Banjoj Luci, Banja Luka, Republika Srpska, Bosna i Hercegovina;
2Katedra za ortopediju vilica, Medicinski fakultet, Studijski program Stomatologija, Univerzitet u Banjoj Luci, Banja Luka, Republika Srpska, Bosna i Hercegovina;
3Univerzitet u Beogradu, stomatološki fakultet, Beograd, Srbija;
4Zavod za kliničku patologiju UKCRS

KRAĐAK SADRŽAJ
Uvod Duboki karijes je reverzibilni proces kod kojeg je karijesna lezija zahvatila veći deo dentina i samo tanak sloj razmekšalog dentina razdvaja leziju od pulpe. Cilj ovog rada je bio da se na početku terapije utvrdi i odrede serotipovi bakterije Aggregatibacter actinomyctesiumcomitans kod zuba sa dubokim karijesnim lezijama. Materijal i metod rada Kliničko ispitivanje je obuhvatalo 29 pacijenata, oba pola, uzrasta od 16 do 40 godina i 45 stalnih zuba kod kojih je na osnovu anamneze, kliničkog i radiografskog pregleda dijagnostikovan duboki karijes. Posle preparacije kavite i uklanjanja razmekšalog dentina, sa dna kavitea je uzimana mala mala količina bakterije Aggregatibacter actinomyctesiumcomitans uzgajena na aerobnoj sredini. Klasifikacija bakterije Aggregatibacter actinomyctesiumcomitans zasnovana je na uvođenju ili određivanju serotipova bakterije Aggregatibacter actinomyctesiumcomitans uzgajene u aerobnoj sredini. Rezultati Serotipizacija je registrovana u 21 uzorku. U jednom od tri uzorka identifikovano je dva serotipa A. actinomyctesiumcomitans – b i c, što je relativno redak nalaz, dok su u drugom i trećem uzorku identifikovani serotipovi a, odnosno serotip b. Zaključak U tri uzorka nađena su tri serotipa – a, b i c, a jedan od uzoraka je nosio čak dva različita serotipa, što je redak fenomen. Za obzirom na epidemiološku i zdravstvenu svrhu serotipizacije bakterije A. actinomyctesiumcomitans na nivou populacije neophodan je neuporedivo veći broj uzoraka.

Ključne reči: duboka karijesna lezija; Aggregatibacter actinomyctesiumcomitans; serotipovi PCR

UVOD
Zubni karijes je hronična kompleksna bakterijska infekcija koja dovodi do miligramskih gubitaka minerala iz zuba koji je zahvaćen infekcijom. Pojedini autori su definisali karijes kao proces deformacije tijela zuba koji nekada ima deo, a danas ima samo nizinu. Karijes je poremećaj u normalnom mineralnom sastojanju i strukturi zuba. Uzrokovani je bakterijama koje producirajte različite enzime, a izazvani su također i drugim razlogima. Klinički je poznat po svojoj multifakturnosti i raznolikosti.

Materijal i metod rada
Kliničko ispitivanje je obuhvatalo 29 pacijenata, oba pola, uzrasta od 16 do 40 godina i 45 stalnih zuba kod kojih je na osnovu anamneze, kliničkog i radiografskog pregleda dijagnostikovan duboki karijes. Posle preparacije kavite i uklanjanja razmekšalog dentina, sa dna kavitea je uzimana mala mala količina bakterije Aggregatibacter actinomyctesiumcomitans uzgajena na aerobnoj sredini. Klasifikacija bakterije Aggregatibacter actinomyctesiumcomitans zasnovana je na uvođenju ili određivanju serotipova bakterije Aggregatibacter actinomyctesiumcomitans uzgajene u aerobnoj sredini. Rezultati Serotipizacija je registrovana u 21 uzorku. U jednom od tri uzorka identifikovano je dva serotipa A. actinomyctesiumcomitans – b i c, što je relativno redak nalaz, dok su u drugom i trećem uzorku identifikovani serotipovi a, odnosno serotip b. Zaključak U tri uzorka nađena su tri serotipa – a, b i c, a jedan od uzoraka je nosio čak dva različita serotipa, što je redak fenomen. Za obzirom na epidemiološku i zdravstvenu svrhu serotipizacije bakterije A. actinomyctesiumcomitans na nivou populacije neophodan je neuporedivo veći broj uzoraka.

Ključne reči: duboka karijesna lezija; Aggregatibacter actinomyctesiumcomitans; serotipovi PCR

Takahashi i saradnici su pokazali na animalnom modelu da SPA serotipa b imaju izraženiji učinak. Studija ponosa i oslobađanje interleukina-1 iz makrofaga od SPA sojeva a i c. Najnoviji podaci zasnovani na molekularno-genetičkim analizama ukazuju na značajnu divergentnu evoluciju genoma bakterija Aggregatibacter actinomyctesiumcomitans. Slično je i u Finskoj populaciji, gde je zadržalo iste bakterijske serotipove, a kod 42% su se pojavili ispitane na početku i na kraju studije (nakon osam godina) 58% stabilan [10]. Međutim, novija istraživanja, zasnovana na molekularno-genetičkim testovima, ukazuju na to da su moguće serotipske promene tokom vremena. U studiji Van der Reijsa i saradnika [11] na stanovništvu indonežanskog ostrva Java, nakon osam godina (vremenski interval studije) došlo je do promene prevalencije serotipova na populacionom nivou. Od osoba koje su ispitane na početku i na kraju studije (nakon osam godina) 58% je zadržalo iste bakterijske serotipove, a kod 42% su se pojavili drugi. Zanimljiva je i činjenica da su uočeni retki slučajevi infekcija učinak multiplnih serotipova (oko 10%), dok je u pomenutoj studiji podaci pokazali da je multiplni serotip dominirao među pacijentima sa parodontopatijama, a i u SAD kod pacijenata sa lokalizovanim juvenilnim periodontitisom češći je serotip b od serotipova a i c. Slično je i u Finskoj populaciji, gde je serotip b dominirao među pacijentima sa parodontopatijama,
dok se serotip c često sreće kod osoba koje su parodontalno zdrave. U japanskoj populaciji kod oboljelih su najučestaliji serotipovi a, c i e [13].

Cilj ovog rada je bio da se na početku terapije utvrde i odrede serotipovi bakterije Aggregatibacter actynomycetemcomitans kod zuba sa dubokim karijesnim lezijama.

MATERIJAL I METOD RADA
Kliničko istraživanje je sprovedeno na 45 stalnih zuba pacijenata dobi od 16 do 40 godina. U istraživanju se bili uključeni stalni zubi različitih morfoloških grupa sa dubokim karijesnim lezijama. Duboka lezija je podrazumijevala karijes zuba kod pacijenata koji je praćen osjetljivosću na termičke nadražaje i koji je zahvatio mali procenat uzoraka mogao da bude serotipski definisan. U ovoj studiji, tokom kliničkog pregleda, takođe je uočena veća prevalencija S. mutans a i L. acidophilus i H. influenzae, a znatno manji efekat P. gingivalis i Porphiromonas gingivalis, a komadi Misvak štapića su ugrađeni u agar podloge.

Oligonukleotidni prajmeri specifični za grupu gena uključenih u biosintezu bakterijskih serotip-specifičnih polisaharidnih antigena dizajnirani su tako da mogu da identificiraju pet glavnih serotipova bakterije Aggregatibacter actynomycetemcomitans (a, b, c, d i e).

Primjeri amplifikacije gena za pojedine serotipove, sa gor- njim parovima prajmera, bili su sledećih dužina: serotip a – 428 bp, serotip b – 258 bp, serotip c – 559 bp, serotip d – 690 bp i serotip e – 211 bp.

Reakcije su rađene u ukupnoj zapremini od 25 mikrolitara, a u laboratorijskim uslovima op- timalnih za izvođenje multipleks PCR-a izgubila se nespecifična traka u uzorku 23 (uzorak 10b). U uzorcima broj 18 i 23 strelicama su označene trake koje ne odgovaraju poznatim serotipovima, a koje bi mog- gle biti artefakti PCR-a.

Jedan od ponovljenih pokušaja serotipizacije uspeo je da identifikuje serotipove u još dva uzorka: 7c i 6. U uzorku 7c serotip je a, a u uzorku 6 serotip je c. Tokom ponovljene reakcije multipleks PCR-a izgubila se nespecifična traka u uzorku 23 koja je bila prisutna u prvom eksperimentu (Slika 2).

DISKUSIJA
Bakterija A. actynomycetemcomitans, odnosno njen serotip c, predstavlja deo normalne flore usne suptilije kod zdravih pacijenata. Ova bakterija može biti i patogena jer poseduje značajan faktor virulencije – adheziju, koja omogućava kolonizaciju bakterija, čime se pojačava njen destruktivni potencijal u oralnim oboljenjima [14].

Serotipizacija bakterija je adekvatna tipizaciona metoda za epidemiološka ispitivanje jer ima mnoge prednosti u odnosu na druge metode ispitivanja bakterija. Prvenstveno je jednostavnija za izvođenje i osetljivija u odnosu na druge metode koje zahtevaju dodatnu opremu i finansijski su zahtevnije [15].

Mnoge bakterije učestvuju u nastanku karijesa pa je i serotipi- zacija kao postupak određivanja pojedinih bakterija vrlo zna- čajna u razjašnjavanju etiologije. Veliki broj istraživanja je poka- zao da su pojedine bakterije koje su detektovane u dentalnom plaku usko povezane sa nastankom karijesa, a velike karijesne lezije često komuniciraju sa bakterijama subgingivalnog biofilma. Streptococcus mutans i Aggregatibacter actynomycetemcomi- tans su oralne patogene bakterije koje se dovode u vezu sa karijesom i sa parodontopatijom. Upravo zbog toga su S. mutans i A. actinomycetemcomitans u uslovima ne samo u ratu nego i u posleratnom periodu na ovim zubima, a kod svih ispitanika je pronađen karijes ili parodontološka pro- mena tokom kliničkog pregleda. Rezultati su pokazali umerenu do visoke prevalence S. mutans i A. actinomycetemcomitans u uslovima [16–20].

Veliki broj zuba zahvaćenih dubokim karijesom i u gornjoj i u donjoj vilici (bez obzira na morfološku grupu zuba) mogao bi se objasniti pre svega socijalnoekonomskim i zdravstvenim uslovima u definitivnom redu. Ipak, najvažniji faktor bi mogao biti stepen zdravstvene zaštite koji ne zadovoljava osnovne zdravstvene potrebe stanovništva. Takođe, teška socijalna situacija u borbi za čistu izgustizenciju često stavlja zdravlje zuba u drugi plan. Češća poja- va dubokog karijesa na molarima gornje i donje vilice mogla bi se objasniti činjenicom da je okluzalna površina ovih zuba pri- jemljava za karijes, zbog njihove morfologije odnosno postojanja fisura i kvržica. Takođe, poznato je da dominantan uticaj na po- java karijesa imaju ishrana, primena fluorida i oralna higijena.

U ovoj studiji, tokom kliničkog pregleda, takođe je uočena veća prevalencija S. mutans a i L. acidophilus i H. influenzae, a znatno manji efekat P. gingivalis i Porphiromonas gingivalis, a komadi Misvak štapića su ugrađeni u agar podloge.

S. mutans i A. actinomycetemcomitans u uslovima [16–20].

Veliki broj zuba zahvaćenih dubokim karijesom i u gornjoj i u donjoj vilici (bez obzira na morfološku grupu zuba) mogao bi se objasniti pre svega socijalnoekonomskim i zdravstvenim uslovima u definitivnom redu. Ipak, najvažniji faktor bi mogao biti stepen zdravstvene zaštite koji ne zadovoljava osnovne zdravstvene potrebe stanovništva. Takođe, teška socijalna situacija u borbi za čistu izgustizenciju često stavlja zdravlje zuba u drugi plan. Češća poja- va dubokog karijesa na molarima gornje i donje vilice mogla bi se objasniti činjenicom da je okluzalna površina ovih zuba pri- jemljava za karijes, zbog njihove morfologije odnosno postojanja fisura i kvržica. Takođe, poznato je da dominantan uticaj na po- java karijesa imaju ishrana, primena fluorida i oralna higijena.

U ovoj studiji, tokom kliničkog pregleda, takođe je uočena veća prevalencija S. mutans a i L. acidophilus i H. influenzae, a znatno manji efekat P. gingivalis i Porphiromonas gingivalis, a komadi Misvak štapića su ugrađeni u agar podloge te je uočen njihov najveći inhibitorni efekat kod P. gingivalis, A. actinomycetemcomitans, H. influenzae, a znatno manji efekat na S. mutans i L. acidophilus [21].
U literaturi se navodi podatak da je za 3 do 8% uzoraka *A. actynomycetemcomitans* nemoguće odrediti serotip [22]. Nažalost, u našoj studiji taj procenat je neuporedivo veći. Pokušaj da se na razne načine prevaziđe tehnički problemi, menjanjem eksperimentalnih parametara, nije dalo rezultate. Multipleks PCR je rađen sa različitom količinom polaznog materijala i u različitom trajanju pojedinih koraka reakcije. Takođe, menjaj je i broj ciklusa (25, 30, 35), a modificovane su i koncentracije MgCl₂ kao i temperature hibridizacije. Međutim, i posle svih napora i ponovljenih pokušaja, uspeh u serotipizaciji je bio veoma skroman. Kod samo 21% uzoraka određen je serotip bakterija *A. actynomycetemcomitans*. Mnogobrojnim studijama se došlo do zaključka da je *A. actynomycetemcomitans* ipak najčešće povezan sa parodontološkim oboljenjima [23].

Cortelli i saradnici su u svom istraživanju (2005) utvrdili zastupljenost *A. actynomycetemcomitans* kod 41,6% ispitanika sa hroničnom parodontopatijom i kod 72% pacijenata sa akutnom parodontopatijom [24].

Takođe, Tinoco i saradnici su pronašli bakteriju *A. actynomycetemcomitans* kod 80% uzoraca mladih ispitanika sa parodontopatijom i predložili da zastupljenost ove bakterije u usnoj šupljini može poslužiti kao indikator rizika za buduća ispitivanja akutne parodontopatije [25].

Simon-Soro i Mira su na osnovu istraživanja dubokih karijesnih lezija otkrili raznovrsan ekosistem, sačinjen od velikog broja bakterija, koji utiče na širenje karijesne lezije. Rezultati su ukazali na to da je *S. mutans* bio zastupljen u manjem procentu, a da veliki broj bakterija, a samim tim i *A. actynomycetemcomitans* ima uticaj na širenje karijesne lezije [26].

**ZAKLJUČAK**

S obzirom na mali uzorak zuba i relativno mali procenat pozitivnih uzoraka *A. actynomycetemcomitans*, odnosno slabiji uspeh multipleks reakcije serotipizacije, teško je govoriti o preveličini pojedinih serotipova u našoj populaciji. U tri uzorka nađena su tri serotipa (a, b i c), a jedan od uzoraka je nosio čak dva različita serotipa, što je redak fenomen. Za ozbiljniju epidemiološku studiju serotipova *A. actynomycetemcomitans* na nivou populacije i njihovu vezu za nastankom karijesa neophodan je neuporedivo veći uzorak, i to reda veličine nekoliko stotina.