SENSITIVITY TO CHITOSAN ASCORBATE MICROAEROPHILIC BACTERIA ISOLATED FROM INFECTIONS OF ORAL CAVITY

Barbara Kochańska[1*], Anna Kędzia[2], Alina Gębska[2]

[1] *Department of Conservative Dentistry, Medical University of Gdańsk, E. Orzeszkowej str. 18, 80-208 Gdańsk, Poland e-mail:bkochan@gumed.edu.pl*

[2] *Department of Oral Microbiology, Medical University of Gdańsk, Do Studzienki str. 38, 80-227 Gdańsk, Poland*

ABSTRACT

The aim of the date was to determine the sensitivity to chitosan ascorbate microaerophilic bacteria isolated from pathological pockets, root canals and apical abscesses. Chitosan was obtained from Antarctic krill. The experiments included 70 clinical isolates and 4 standards strains. The susceptibility of microaerophilic bacteria was performed by means of plate dilution technique in Brucella agar supplemented with 5% defibrinated sheep blood. Inoculum contained 10^5 CFU per spot. Incubation inoculated agar was performed at 37°C for 48 hrs in microaerophilic conditions. MIC was defined as the least concentrations of chitosan ascorbate that inhibited growth of tested bacteria. The results indicated that 47% of all date bacteria was susceptible to chitosan ascorbate in ranges ≤ 0.01 – 0.5 mg/ml. The most susceptible were the strains of Corynebacterium matruchotii (100%, MIC ≤ 0.01 – 0.5 mg/ml) and the least sensitive strains of Rothia dentocariosa (MIC in ranges 1.0 - ≥ 4.0 mg/ml).

Key words*: microaerophilic bacteria, chitosan ascorbate, susceptibility, oral cavity*

Received: 07.03.2016
Accepted: 24.05.2016

1. Introduction

Five hundred a variety microorganisms have been isolated from the oral cavity. Certain bacterial genus are constantly found in the specific oral areas. These bacterial genus are referred to collectively as the normal, indigenous or commensal microflora and constitute the oral ecosystem [1, 2, 3, 4]. The microorganisms can grow in the presence (aerobic) or absence of oxygen (anaerobic). In addition, there are some capnophilic (CO_2 – requiring) and microaerophilic bacteria which requiring low concentrations of oxygen for its growth. Among the genus of oral cavity there are *Aggregatibacter* (formerly *Actinobacillus*), *Capnocytophaga*, *Campylobacter*, *Corynebacterium* (some strains), *Eikenella* and *Rothia*. *Aggregatibacter actinomycetemcomitans* (Gram-negative rods) has been implicated in the aetiology of particularly destructive form of periodontal disease in adolescents [1, 2, 3, 5, 6, **7**]. This bacterium possess a fimbriae and surface layers that stimulate bone resorption. It produces a variety of virulence factors, including leukotoxin, collagenase and proteases capable of cleaving IgA [5, 6, 7]. The *Capnocytophaga* are Gram-negative rods. They produce an IgA protease [8, 9, 10]. This genus includes following species: *Capnocytophaga ochracea*, *C. sputigena*, *C. haemolytica*, *C. granulosa* and *C. gingivalis*. These bacteria are opportunistic pathogens and have been isolated from infections in immunocompromised patients. *Capnocytophaga* strains have been found in *subgingival* plaque and *gingivitis* [2, 8, 9, 10]. The *Eikenella corrodens* are Gram-negative small rods. Colonies of this species characteristically pit the surface agar. The rods has been isolated from varietal oral infections, e.g. *gingivitis*, *periodontitis* and *abscesses* [2, 11, 12, 13, 14]. This microorganism also can cause subacute bacterial *endocarditis*.

The *Campylobacter* are Gram-negative rods (cells have a spiral morphology) [2, 3, 11, 14, 15]. They include species e.g. *Campylobacter concisus*, *C. showae*, *C. sputorum* and *C. gracilis*. The rods have two to five flagella, only *Campylobacter rectus* has a single polar flagellum. This bacteria often has been detected in large numbers in deeper *subgingival* pockets and has been proposed to play a pathogenic role in human *periodontitis* [16]. Surface components, such as the flagellum and cytotoxin have been reported as possible major pathogenic factors of the rods.

Corynebacterium matruchotii are irregular Gram-positive no motile, nonsporulating rods, with whip-handle shape and branching filaments. These bacteria are found in the oral cavity, especially in calculus and dental plaque [3]. They are able to form intracellular calcification.

Rothia dentocariosa is Gram-positive rods, which appears as a mixture of branched filaments and coccoid cells. These bacteria are found in oral cavity, particularly in dental plaque [3, 15]. They are isolated from variety infections, such as chest abscess, throat abscess, postoperative wounds and infective *endocarditis*.

The aim of the study was to determine the sensitivity to chitosan ascorbate microaerophilic bacteria isolated from infections of oral cavity.

2. Materials and Methods

Materials were taken from 70 patients with infections in oral cavity. The investigations included 70 strains of microaerophilic bacteria isolated from pathological pockets (27 strains), root canals (30 strains) and apical abscesses (13 strains). The following bacterial strains were tested: *Aggregatibacter* (22 strains), *Corynebacterium* (3 strains), *Capnocytophaga* (5 strains), *Campylobacter* (19 strains), *Eikenella* (17 strains), *Rothia* (4 strains) and four standard strains; *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Chitosan was obtained from Antarctic krill. The preparation included chitosan and ascorbic acid was prepared in Sea Fisheries Institute in Gdynia (Poland). The ratio of ascorbic acid to chitosan was equal to 1. The degree of deacetylation was equal to 60%.

Right before the experiment, chitosan ascorbate was dissolved in a sterile distilled water. The following concentrations were obtained: 4.0, 2.0, 1.0, 0.5, 0.25, 0.12, 0.06, 0.03 and 0.01 mg/ml. The susceptibility of microaerophilic bacteria was performed by means of the plate dilution techniques in Brucella agar with addition of 5% defibrinated sheep blood. Inoculum containing 10^5 CFU per spot was seeded with Steers replicator upon the surface of the agar with and without chitosan ascorbate (bacterial growth control). Incubation inoculated plates was performed at 37°C for 48 hrs in anaerobic jars with Campy Pak (BBL). MIC was defined as the least concentrations of chitosan ascorbate that inhibited growth of tested bacterial strains.

3. Results and Discussion

Table 1 demonstrates sensitivity microaerophilic bacteria and Table 2 standards strains to graded concentrations of chitosan ascorbate used in the study. The data indicated that chitosan ascorbate exhibit different antimicrobial activity. From all tested microaerophilic bacteria 33 (47%) strains were susceptible to tested preparation in low concentrations, in ranges ≤ 0.01 – 0.5 mg/ml. From among examined the Gram-negative rods the growth of 30 (48%) strains was inhibited by chitosan ascorbate in concentrations in the range ≤ 0.01 – 0.5 mg/ml. The most susceptible were the strains from genus of *Aggregatibacter* and *Capnocytophaga*. The growth of 16 (72%) and 2 (40%) strains respectively was inhibited by concentrations within the range of ≤ 0.01 – 0.5 mg/ml. In our previous study the *Aggregatibacter actinomycetemcomitans* strains were similar susceptible [17]. Chitosan ascorbate was active in concentrations from ≤ 0.06 to ≥ 2.0 mg/ml against 77% tested strains. In our other dates [18] the strains of *A. actinomycetemcomitans* was more sensitive. The tested preparation on the range of concentrations between ≤ 0.06 and 2.0 mg/ml inhibited growth of 83% of strains.

Table 1. Susceptibility of 70 strains of microaerophilic bacteria to chitosan ascorbate

Microorganisms	**Number of strains**	**Minimal inhibitory concentration MIC mg/ml**								
		≥4.0	2.0	1.0	0.5	0.25	0.12	0.06	0.03	≤0.01
Gram-negative rods: *Aggregatibacter actinomycetemcomitans*	22	5	1		2	1	1		3	9
Capnocytophaga gingivalis	5	3						1		1
Campylobacter sputorum	10	6	1		2	1				
Campylobacter gracilis	9	4	2						1	2
Eikenella corrodens	17	5	6		1	1	1		1	2
Gram-negative rods Total	**63**	**23**	**10**		**5**	**3**	**2**	**1**	**5**	**14**
Gram-positive rods: *Corynebacterium matruchotii*	3				1				1	1
Rothia dentocariosa	4	2	1	1						
Gram-positive rods Total	**7**	**2**	**1**	**1**	**1**				**1**	**1**
Microaerophilic bacteria Total	**70**	**25**	**11**	**1**	**6**	**3**	**2**	**1**	**6**	**15**

Table 2. Susceptibility of standards strains to chitosan ascorbate

Microorganisms	Number of strains	Minimal inhibitory concentration MIC mg/ml								
		≥4.0	2.0	1.0	0.5	0.25	0.12	0.06	0.03	≤0.01
Escherichia coli ATCC 25922	1	1								
Enterococcus faecalis ATCC 29212	1	1								
Staphylococcus aureus ATCC 25923	1		1							
Pseudomonas aeruginosa ATCC 27853	1	1								

In our other dates [18] the strains of *Campylobacter spp.* were the most susceptible than strains in our present experiments. The growth of all strains *Campylobacter spp*. was inhibited by chitosan ascorbate concentrations within the range of ≤ 0.06 – 0.5 mg/ml [18]. In the present study only 32% examined strains of *Campylobacter spp.* was inhibited by the same concentrations.

The strains of *Eikenella corrodens* were less susceptible. The preparation in low concentrations from ≤ 0.01 to 0.5 mg/ml inhibited growth of 35% strains. In our previous dates [17] the strains from genus *Eikenella* were more susceptible in the same concentrations - chitosan ascorbate were active against 67% of the strains.

Among Gram-positive rods, the strains *Corynebacterium matruchotii* showed high degree of sensitivity to tested preparation. All strains were susceptible at concentrations from ≤ 0.01 to 0.5 mg/ml. The strains of *Rothia dentocariosa* were the lowest sensitive (MIC in ranges from 1.0 to ≥ 4.0 mg/ml).

4. Conclusions

Chitosan ascorbate showed good activity against microaerophilic bacteria. MIC 47% of strains was from ≤ 0.01 to 0.5 mg/ml. The most susceptible were *Corynebacterium matruchotii* (100% susceptible) and less the strains of *Aggregatibacter actinomycetemcomitans* (72% susceptible). The Gram-negative bacteria were somewhat more sensitive to chitosan ascorbate than Gram-positive ones (48% and 43% strains susceptible, respectively). The strains of *Capnocytophaga gingivalis* were the lowest sensitive (MIC ≤ 0.01 – 0.06 mg/ml inhibited growth of 40% the strains). The least sensitive showed strains of *Rothia dentocariosa* (MIC 1.0 - ≥ 4.0 mg/ml)

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