

THE SUSCEPTIBILITY TO ANTIBIOTICS OF SOME *STREPTOCOCCUS CONSTELLATUS* STRAINS ISOLATED FROM ODONTOGENIC INFECTIONS

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Abstract

The aim of the present study was the investigation of the antimicrobial susceptibility of 45 isolates of *Streptococcus constellatus* from different odontogenic infections. The E-test was applied for testing their susceptibility against the following antibiotics: penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin, tetracycline, levofloxacin, linezolid and vancomycin. In addition, the double disk diffusion method was used for determining the erythromycin resistance phenotype. The results indicated that all the strains were susceptible to: beta-lactam antibiotics, levofloxacin, linezolid and vancomycin, while only 56% of them were susceptible to tetracycline. Six of the tested strains were resistant to erythromycin, showing the M phenotype, except for one isolate which manifested inducible clindamycin resistance. In conclusion, penicillin remains the therapeutic choice when *S. constellatus* is involved in infections requiring antimicrobial treatment, but in case of allergy to beta-lactam antibiotics, macrolides and clindamycin should be chosen as alternatives only, according to the *in vitro* susceptibility results.

Rezumat

Scopul prezentului studiu l-a reprezentat investigarea sensibilității la antibiotice a 45 de izolate de *Streptococcus constellatus*, provenind din diferite infecții odontogene. A fost aplicat E-test-ul pentru testarea sensibilității acestora față de următoarele antibiotice: penicilina G, ampicilină, cefotaxim, eritromicină, clindamicină, tetraciclină, levofloxacin, linezolid și vancomicină. Pe lângă aceasta, a fost utilizată metoda difuzimetrică a dublului disc în vederea determinării fenotipului de rezistență la eritromicină. Rezultatele au indicat faptul că toate tulpinile au fost sensibile la: beta-lactamine, levofloxacin, linezolid și vancomicină, în timp ce doar 56% dintre acestea au fost sensibile la tetraciclină. Șase dintre tulpinile testate au fost rezistente la eritromicină, prezentând fenotipul M, cu excepția unui singur izolat, ce a manifestat rezistență inductibilă la clindamicină. În concluzie, penicilina rămâne antibioticul de elecție pentru infecțiile în care este implicat *S. constellatus*, atunci când acestea necesită tratament antimicrobian, însă, în caz de alergie la beta-lactamine, macrolidele și clindamicina ar trebui alese ca alternative numai în conformitate cu rezultatele investigației sensibilității *in vitro*.

Keywords: *Streptococcus constellatus*, odontogenic infections, antibiotic resistance

Introduction

The pyogenic diseases of dental origin are mainly mixed infections, involving usually both aerobic and anaerobic microorganisms, with the last ones predominating [7, 8, 10]. The oral streptococci are the most frequently facultative anaerobic bacteria isolated from odontogenic infections, especially the species belonging to the *anginosus* group. This group, formerly called *S. milleri* group, comprises 3 species: *S. anginosus*, *S. intermedius* and *S. constellatus*. The last species contains 2 subspecies: *S. constellatus* subsp. *pharyngis* and *S. constellatus* subsp. *constellatus* [8]. Although *S. constellatus* belongs to the normal oral flora, it may manifest its pathogenic potential when entering in normally sterile areas, through dental and surgical interventions, invasive diagnostic and treatment measures, or

local trauma. It has been isolated, especially from immunocompromised patients, from different kind of infections: rhinosinusitis, pharyngitis, pneumonia, pulmonary abscess, thoracic empyema, cystic fibrosis, brain abscess, meningitis, subdural empyema, infective endocarditis, liver abscess, pancreatic abscess, splenic abscess and various other infections [6, 7, 8].

S. constellatus is involved (mainly in association with other bacteria, especially with the anaerobes) in many purulent odontogenic infections. The pyogenic diseases of dental origin are very frequently met in Romanian patients and sometimes require also antibiotic treatment, in addition to surgical incision and drainage. For this reason, the aim of this study was to investigate the *in vitro* antibiotic susceptibility of some *S. constellatus* strains isolated from Romanian patients with different types of odontogenic infections.

Materials and Methods

In the present study, the 45 non-duplicate isolates of *S. constellatus* tested for their antimicrobial susceptibility belonged to a collection of isolates stored in cryo-bead tubes (AES Laboratoire, France), at -70°C, at the Department of Microbiology laboratory, Faculty of Dentistry, “Carol Davila” University of Medicine and Pharmacy, Bucharest. These strains had been isolated from pus samples collected by needle aspiration from patients with different odontogenic infections (oral and maxillofacial abscesses, sialadenitis, pericoronitis etc.), presented to the Dental University Hospital, Bucharest, and at the Private Dental Practice “Dr. Mircea Pănculescu”, Bucharest, during February 2009 - February 2012. These isolates had been previously identified at species level by the Rapid ID 32 STREP system (BioMérieux, France).

In the present research work, the 45 isolates were tested for their susceptibility to 9 antibiotics: penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin, tetracycline, levofloxacin, linezolid and vancomycin by the E-test (BioMérieux, France). The reference strain *S. pneumoniae* ATCC 49619 was used as quality control. The bacterial inoculum made in Müller-Hinton broth (Oxoid, UK) was standardized at 0.5 McFarland turbidity and swabbed on the surface of Müller-Hinton agar supplemented with 5% sheep blood (BioMérieux, France). After the implementation of E-test strips with the respective antibiotics, they were displayed on the inoculated agar, the Petri plates were incubated in jars with 5% CO₂ atmosphere generated by GENbox CO₂ envelopes (BioMérieux, France), at 35°C, for 24 h. The values of the minimum inhibitory concentrations (MIC) were interpreted according to the breakpoint concentrations recommended by the Clinical and Laboratory Standards Institute (CLSI) [3]. In addition, the macrolide resistance phenotype was investigated by the double disk diffusion test, using erythromycin

(15 µg) and clindamycin (2 µg) disks (Oxoid, UK) placed at a distance of 12 mm each other's.

Results and Discussion

The minimum inhibitory concentration (MIC) values of the 9 antibiotics are presented in Table I. All the isolates were susceptible to the following 6 antibiotics: penicillin G, ampicillin, cefotaxime, levofloxacin, linezolid and vancomycin. Twenty isolates were not susceptible to tetracycline, 5 strains being intermediate susceptible and 15 strains resistant. Five of these last isolates were resistant to both tetracycline and erythromycin. The MIC values of this macrolide were 3 mg/L for 2 strains and 4 mg/L for the other 3 strains, and according to the results of the double disk diffusion method these 5 isolates manifested the M phenotype. Another strain, resistant only to erythromycin (with a MIC value of more than 256 mg/L), showed inducible clindamycin resistance. The percentage distribution of the *S. constellatus* isolates referred to the 3 categories of sensitivity groups is shown in Figure 1.

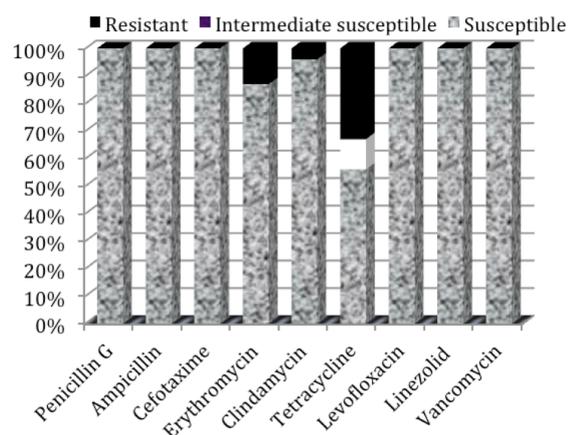


Figure 1. Distribution (%) of the *S. constellatus* isolates by antibiotic susceptibility

Table I
Values of the minimum inhibitory concentrations (MIC)

ANTIBIOTIC	MIC VALUE (mg/L)			
	MIC _{min} ¹	MIC _{max} ²	MIC _{50%} ³	MIC _{90%} ⁴
Penicillin G	0.008	0.12	0.032	0.094
Ampicillin	0.016	0.125	0.023	0.064
Cefotaxime	0.012	0.125	0.023	0.032
Erythromycin	0.016	> 256	0.032	3
Clindamycin	0.016	0.064	0.023	0.032
Tetracycline	0.047	64	0.38	32
Levofloxacin	0.094	0.75	0.38	0.50
Linezolid	0.125	0.75	0.38	0.50
Vancomycin	0.125	0.75	0.50	0.75

¹MIC_{min} = the lowest MIC value in the respective series of strains, ²MIC_{max} = the highest MIC value in the respective series of strains, ³MIC_{50%} = the MIC value that inhibited 50% of the strains, ⁴MIC_{90%} = the MIC value that inhibited 90% of the strains.

In odontogenic infections, the antibiogram is not routinely performed and usually, the antibiotics are empirically administered, when they are required in addition to incision and drainage. However, it is important to test periodically the antibiotic susceptibility of the isolates with clinical significance isolated also from oral and maxillofacial infections. *S. constellatus* and the other 2 species of *anginosus* group are frequently isolated from oral pyogenic infections.

In the present study, the results indicated that all isolates were susceptible to the tested beta-lactam antibiotics, levofloxacin, linezolid and vancomycin, and this is in accordance with the findings of most researchers. However, some recent studies reported *S. constellatus* isolates resistant to penicillin. Thus, Concistrè *et al.* isolated a strain of *S. constellatus* (from the blood culture of a patient who developed infective endocarditis), which was susceptible to many antibiotics (cefotaxime, ceftriaxone, erythromycin, tetracycline, ofloxacin, levofloxacin, chloramphenicol and vancomycin), but resistant to penicillin G [4].

In patients allergic to beta-lactam antibiotics, erythromycin is still considered the drug of choice, but the 13% erythromycin resistant isolates found in this study and the possibility of manifesting inducible resistance to clindamycin indicated that macrolide and clindamycin must be administered only after the *in vitro* susceptibility testing of the respective isolates. The polymerase chain reaction became an important method for detecting various microbial genes [1, 2], including the antibiotic resistance genes [9]. In streptococcal isolates resistant to erythromycin, this molecular technique is applied to detect the macrolide resistance genes and the results are correlated with the following resistance phenotypes: constitutive macrolide-lincosamide-streptogramin (cMLS) phenotype, inducible MLS (iMLS) phenotype or M phenotype [5].

Conclusions

In infections produced by *S. constellatus*, penicillin is still considered the drug of choice. Because odontogenic pyogenic diseases are usually mixed infections (involving both facultative anaerobic and strictly anaerobic microorganisms, some of them being beta-lactamase producers) an association of penicillin with a beta-lactamase inhibitor should be

recommended when the antibiotic treatment is required and it is prescribed empirically.

Tetracycline must be avoided in infections with oral streptococci. In patients with penicillin allergy, the macrolides or clindamycin should be given when the isolates have been proven to be susceptible *in vitro* to these antibiotics.

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