

Antibiotic susceptibility of the bacteria causing odontogenic infections

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ABSTRACT

Aims: An evaluation is made of bacterial species and susceptibility to various antibiotics used in application to odontogenic infections of periapical location and in pericoronitis of the lower third molar, with the aim of optimizing the antibiotherapy of such infections and thus preventing unnecessary side effects and over-treatment.

Material and methods: Sixty-four patients with odontogenic infection were selected on the basis of a series of inclusion and exclusion criteria. Samples were collected from lesions under maximally aseptic conditions, avoiding oral saprophytic contamination. The samples were cultured and incubated under aerobic and anaerobic conditions, followed by bacteriological identification and antibiotic susceptibility testing.

Results: A total of 184 bacterial strains were isolated and identified, comprising grampositive facultative anaerobes (68%), gramnegative strict anaerobes (30%) and grampositive facultative anaerobes (2%). Regardless of the origin of the odontogenic infection, the causal bacteria yielded the best results in terms of increased sensitivity and lesser resistance with amoxicillin / clavulanate and amoxicillin, respectively ($p < 0.05$).

Discussion: There are increasingly numerous reports in the literature of growing bacterial resistance to antibiotics in infectious processes affecting non-buccodental territories. This same tendency has not been observed in relation to oral infections, though important resistance has been documented for certain concrete antibiotics. According to our results, the common-use antibiotics with the greatest sensitivity and lowest resistance were shown to be amoxicillin / clavulanate followed by amoxicillin alone.

Key words: *Odontogenic infection, oral bacteriology, periapical lesion, third molar pericoronitis, antibiotic susceptibility.*

RESUMEN

Objetivos: Identificar la flora bacteriana y su susceptibilidad a varios antibióticos utilizados en infecciones odontogénicas de localización periapical y en las pericoronaritis del tercer molar inferior, para poder adaptar convenientemente el tratamiento antibiótico a las exigencias de tales infecciones, y evitar así los efectos secundarios y los sobretratamientos con antibióticos.

Material y métodos: Se han seleccionado con unos criterios de inclusión y de exclusión a 64 pacientes que presentaban una infección odontogénica. Se recogieron muestras de las lesiones en condiciones de máxima asepsia, evitando la contaminación por flora saprófita bucal. Las muestras se sembraron en medios de cultivo apropiados y se incubaron en condiciones aeróbicas y anaeróbicas; finalmente se procedió a la identificación de los microorganismos aislados y a la determinación de su susceptibilidad antibiótica, los resultados se analizaron estadísticamente mediante la prueba t-Student (para muestras aparejadas y para una muestra).

Resultados: Se aislaron un total de 184 cepas bacterianas, incluyendo cocos Gram positivo anaerobios facultativos (68%), bacilos Gram negativo anaerobios estrictos (30%), y bacilos Gram positivo anaerobios facultativos (2%). Independientemente del origen de la infección odontogénica los antibióticos que obtuvieron los mejores resultados en cuanto a mayor sensibilidad y menor resistencia estadísticamente significativos fueron respectivamente la amoxicilina/clavulánico y la amoxicilina ($p < 0,05$).

Discusión: Cada vez hay más estudios que indican el alto índice de resistencias a antibióticos en poblaciones bacterianas patógenas que producen infecciones en territorios no bucodentales. A pesar de ello, los niveles de resistencia a los antibióticos en las infecciones odontogénicas no han seguido la misma tendencia, aunque se ha detectado para ciertos antibióticos un alto índice de resistencia. En nuestro trabajo hemos encontrado que los antibióticos de uso común que han obtenido mayor sensibilidad y menor resistencia han sido la amoxicilina en combinación con ácido clavulánico seguido de la amoxicilina.

Palabras clave: Infección odontogénica, bacteriología bucal, lesión periapical, periconaritis del tercer molar, susceptibilidad antibiótica.

INTRODUCTION

Although the incidence of odontogenic infections has decreased in recent years as a result of improvements in orodental and general health care, no specific data on their incidence in the general population are available. It is known that odontogenic infections are not caused by a single organism. Indeed, polymicrobial infections are frequently encountered, and in some cases up to 6 different species have been isolated (1-4). Many of the organisms isolated from samples appear to play no relevant pathogenic role, though their presence suggests that they could collaborate in the infectious process by supplying nutrients or growth factors, creating favorable pH conditions, or simply antagonizing other microorganisms (5,6).

The treatment of odontogenic infections is based on two fundamental elements: mechanical-surgical management and antibiotherapy (2). In some cases, antibiotic prescription is empirical and based on the clinical condition of the patient. As a result, treatment is often inappropriate and leads to the development of bacterial resistance and even multiple resistance (7).

Among other causes, odontogenic infections are produced by pericoronaritis and periapical lesions – the origins of which are well known. The types of pathogens found in such lesions are also known, and the management strategies according to the phase of the odontogenic infection have been defined. Antibiotic treatment is needed in most patients with odontogenic infectious processes.

The aim of the present study is to determine which antibiotics should be prescribed in first place in patients with odontogenic infection, and to establish whether different antibiotic regimens are indicated according to whether pericoronaritis or apical lesions are involved. The study also reports the results relating to antibiotic susceptibility of the bacteria responsible for odontogenic infections treated in the Barcelona University Dental Clinic (Spain).

MATERIAL AND METHODS

Over a 14-month period (2001-2002), a total of 64 patients with acute odontogenic infection of pulp origin or associated to lower third molar pericoronaritis were selected in the Barcelona University Dental Clinic, within the context of the health care activity of the Master in Oral Surgery and Implantology.

The included patients were adults (over 18 years of age) not subjected to antibiotherapy in the previous 30 days, with third

molar pericoronaritis or periapical alterations in the acute phase. Patients with an impacted lower third molar and pericoronaritis were required to present a partially erupted molar defined according to the Pell and Gregory classification as corresponding to class IA, IB, 2A, 2B. Furthermore, patients with periapical lesions were required to present an X-ray image consistent with radicular granuloma or cyst.

Patients performing antiseptic mouthrinses in the previous 24 hours were excluded, as were pregnant or nursing women, and patients with gastrointestinal, liver or kidney disease, neoplastic processes or AIDS. A complete clinical history was compiled and a locoregional physical examination was made, with an X-ray study and the application of a specific surgical protocol for third molar inclusions or periapical disease.

Sampling was performed in a surgical setting, after washing the surgical area with 20 ml of sterile saline and continuously aspirating saliva. In the case of patients presenting a purulent exudate due to pericoronaritis or a fistulized periapical lesion, two sterile paper tips (Number 30, Maillefer Dentsply, Ballaigues, Switzerland) were consecutively inserted for subsequent microbiological study.

Patients with an abscess or cellulites were subjected to puncture-aspiration sampling prior to debridement surgery or antibiotic therapy, as applicable. The rest of patients were included in the following surgical protocol: In the case of pericoronaritis due to impacted lower third molars, the bacterial plaque was removed from the surface of the second and third molars with the aid of sterile cotton swabs, after abundant irrigation with saline; a mucoperiosteal flap was raised, luxation and avulsion of the third molar was performed, and the pathological tissue located distal to the third molar was excised. The samples were collected after lesion exposure using sterile paper tips, and were seeded in culture media at that moment in the operating room.

In patients with periapical lesions where the tooth could be preserved, a mucoperiosteal flap was raised followed by ostectomy, excision of the periapical lesion, apicoectomy and retrograde filling – with the collection of a root canal sample for culture. The sampling procedure used was the same as in the case of pericoronaritis. Removal with excision of the periapical lesion was decided in the case of teeth that could not be preserved due to reabsorption of over one-third of the root or severe periodontal disease. Such extraction was also decided in the case of non-restorable teeth, complete cortical loss, and in cases where the patient did not accept any other type of treatment.

Sampling was again performed as in the case of patients with pericoronaritis and periapical lesions.

All collected samples (paper tips, puncture-aspiration) and pathological tissues were collected intraoperatively and cultured directly in sheep blood agar plates (Biomérieux, Lyon, France). Two plates were seeded for each sample: one was incubated in contact with air at 37°C, while the other was incubated in BBL Gaspack System jars (Decton Dickinson, USA) using Anaerogen® Oxoid to ensure anaerobiosis (Hampshire, England).

The isolates obtained were tested against the following antibiotics: amoxicillin, amoxicillin / clavulanate, erythromycin, metronidazole, tetracycline, clindamycin, azithromycin, and linezolid. Antibiotic susceptibility was established by measuring the minimum inhibitory concentration (MIC) by microdilution in liquid medium. The MIC values indicating resistance or susceptibility of each of the antibiotics were based on the reference criteria of the National Committee for Clinical Laboratory Standards (NCCLS).

The antibiotic solutions were prepared in the laboratory by dissolution of pure products (Sigma-Aldrich Chemical Co., St. Louis, USA). From the initial solutions, serial dilutions of each antibiotic were prepared (1:2) from 32 µg/ml to 0.008 µg/ml in culture medium. The dilutions were prepared in 96-well microtitration plates and were inoculated with 24-hour culture diluted 100 times. The plates were incubated for 24 hours at 37°C, after which plate turbidity was assessed visually. The antibiotic concentration in the well with the lowest concentration where no turbidity was seen was interpreted as the MIC value of the antibiotic for the bacterium involved. The plates were in turn also evaluated using an ELISA reader (Boehringer EL 311 Microplate Reader, Barcelona, Spain) to confirm the visual results.

The student t-test for paired samples was used to compare resistance versus sensitivity, resistance-resistance and sensitivity-sensitivity of the strains with all the antibiotics tested. The Student t-test for a single sample was applied, with test values in excess of 25% for resistances, and of under 75% in the case of sensitivity.

RESULTS

The study comprised 39 men (60.9%) and 25 women (39.1%), with a mean age of 40.5 years (range 18-63). Of the 64 patients studied, 43 (67%) had lower third molar pericoronitis and 21 (33%) presented periapical disease.

The most frequent clinical manifestations in patients with pericoronitis were local pain (n = 41; 95.3%), swelling (n = 36; 83.7%), trismus (n = 13; 30.2%), seropurulent exudate (n = 7; 16.3%), abscess (n = 6; 14%), cellulites (n = 4; 9.4%) and fever (n = 2; 4.7%).

In patients with periapical disease, the most frequent clinical manifestations were local pain (n = 14; 66.7%), fistulization of the lesion (n = 5; 23.8%), abscess (n = 3; 14.3%), seropurulent exudate (n = 3; 14.3%), and cellulites (n = 1; 4.8%).

A total of 184 bacterial strains were isolated (2-5 pathogens from each sample). Identification classified the bacteria by genus and species in all cases. The prevalent isolates were

grampositive and mainly fermentative cocci (*Enterococcus faecalis* and *Streptococcus mutans* and *oralis*). Of the grampositive cocci, *Streptococcus* was the microorganism most frequently colonizing lower third molar pericoronitis (54.4%), while *Enterococcus* was associated with periapical lesions (19.2%)(Table 1). In this context, Table 1 shows the relative and absolute values corresponding to the rest of bacterial strains, and their relation to the type of pathology diagnosed.

Bacterial susceptibility to the different antibiotics is summarized in Tables 2 and 3. In absolute terms, the strains were seen to be highly sensitive to the more commonly used antibiotics (amoxicillin / clavulanate and amoxicillin alone), and to linezolid. In contrast, important resistance to metronidazole was observed.

In the study of antibiotic susceptibility according to the severity of the clinical condition, high bacterial sensitivity to amoxicillin, amoxicillin / clavulanate and linezolid was observed, regardless of the severity of the condition and the origin of the odontogenic infection.

Statistically, on comparing the antibiotics in terms of sensitivity-resistance, all drugs except erythromycin, azithromycin and metronidazole were seen to exhibit greater sensitivity than resistance (p<0.05). On comparing only resistance among the different antibiotics, the lowest resistance values were seen to correspond to amoxicillin and linezolid (p<0.05). In turn, amoxicillin showed significantly superior sensitivity versus tetracycline and metronidazole (p<0.05), though not versus the rest of the drugs studied (p>0.05). On contrasting resistance with a maximum 25% cutoff value, amoxicillin, amoxicillin / clavulanate and linezolid were seen to exhibit significantly lower resistance than the rest of drugs (p<0.05), while for a minimum 75% cutoff value only amoxicillin and amoxicillin / clavulanate exceeded 75% (p<0.05).

DISCUSSION

The normal bacterial microflora of the oral cavity comprises mainly anaerobic bacteria. It is therefore not surprising that studies of odontogenic infections find the prevalence of anaerobic bacteria to be higher in dentoalveolar infectious processes (8,9).

In some studies of odontogenic infection, grampositive facultative anaerobic cocci of the genus *Streptococcus* remain the most frequent microorganisms, while in other studies the greatest prevalence corresponds to gramnegative strict anaerobic bacilli represented by black-pigmented species of the genera *Porphyromonas* and *Prevotella*, closely followed by grampositive strict anaerobic cocci of the genus *Peptostreptococcus* and gramnegative strict anaerobic bacilli of the genus *Fusobacterium* (2).

According to Herrera et al. (10) in their study of periodontal abscesses, *Fusobacterium nucleatum* is seen to predominate. Other authors report that an increased presence of strict anaerobes is seen in periapical lesions, in agreement with our own findings (1,11-13).

As in our study, a number of investigators have found lower third molar pericoronitis to be mainly associated with grampositive facultative anaerobic cocci of the genus *Streptococcus*,

Table 1. Bacteriological analysis of periapical lesions and lower third molar pericoronitis.

| | Pericoronitis (no. strains, %) | Periapical lesion (no. strains, %) | Total (no. strains, %) |
|---|--|--|----------------------------------|
| Grampositive facultative anaerobic cocci (125 strains) | | | |
| <i>Streptococcus mutans, oralis</i> | 68 (54.4%) | 22 (17.6%) | 90 (72%) |
| <i>Enterococcus faecalis</i> | 11 (8.8%) | 24 (19.2%) | 35 (28%) |
| Gramnegative strict anaerobic bacilli (55 strains) | | | |
| <i>Bacteroides forsythus</i> | 7 (12.8%) | 26 (47.3%) | 33 (28%) |
| <i>Fusobacterium nucleatum</i> | 5 (9.1%) | 7 (12.6%) | 12 (21.8%) |
| <i>Porphyromonas gingivalis</i> | 2 (3.6%) | 4 (7.3%) | 6 (10.9%) |
| <i>Prevotella intermedia</i> | 1 (1.8%) | 3 (5.5%) | 4 (7.2%) |
| Grampositive facultative anaerobic bacilli (4 strains) | | | |
| <i>Actinomyces actinomycetemcomitans</i> | 1 (25%) | 3 (75%) | 4 (100%) |

Table 2. Distribution of antibiotic susceptibility according to bacterial group.

R = Resistant, S = Sensitive, I = Intermediate

| | | Gram + cocci (no. strains, %) | Gram – bacilli (no. strains, %) | Gram + bacilli (no. strains, %) | Total (no. strains, %) |
|--------------------------------|---|---|---|---|----------------------------------|
| Erythromycin | R | 49 (39.2%) | 21 (38.2%) | 2 (50%) | 72 (39.1%) |
| | S | 68 (54.4%) | 32 (58.2%) | 1 (25%) | 101 (54.9%) |
| | I | 8 (6.4%) | 2 (3.6%) | 1 (25%) | 11 (6%) |
| Tetracycline | R | 10 (8%) | 17 (30.9%) | 1 (25%) | 28 (15.2%) |
| | S | 115 (92%) | 38 (69.1%) | 3 (75%) | 156 (84.8%) |
| | I | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Amoxicillin | R | 6 (4.8%) | 12 (21.8%) | 0 (0%) | 18 (9.8%) |
| | S | 119 (96%) | 43 (78.2%) | 4 (100%) | 166 (90.2%) |
| | I | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Amoxicillin/clavulanate | R | 5 (4%) | 12 (21.8%) | 0 (0%) | 17 (9.2%) |
| | S | 120 (96%) | 43 (78.2%) | 4 (100%) | 167 (90.8%) |
| | I | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Metronidazole | R | 42 (33.6%) | 47 (85.5%) | 4 (100%) | 93 (50.5%) |
| | S | 83 (66.4%) | 8 (14.5%) | 0 (0%) | 91 (49.5%) |
| | I | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Clindamycin | R | 25 (20%) | 10 (18.2%) | 1 (25%) | 36 (19.6%) |
| | S | 100 (80%) | 45 (81.8%) | 3 (75%) | 148 (80.4%) |
| | I | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Azithromycin | R | 39 (31.2%) | 21 (38.2%) | 1 (25%) | 61 (33.2%) |
| | S | 76 (60.8%) | 33 (60%) | 2 (50%) | 111 (60.3%) |
| | I | 10 (8%) | 1 (1.8%) | 1 (25%) | 12 (6.5%) |
| Linezolid | R | 1 (0.8%) | 2 (3.6%) | 1 (25%) | 4 (2.2%) |
| | S | 122 (97.6%) | 50 (91%) | 2 (50%) | 174 (94.6%) |
| | I | 2 (1.6%) | 3 (5.4%) | 1 (25%) | 6 (3.2%) |

Table 3. Distribution of antibiotic susceptibility according to the bacterial species involved. Number of strains (%).
R = Resistant, S = Sensitive, I = Intermediate

| | | <i>E. faecalis</i> | <i>S. mutans, oralis</i> | <i>B. forsythus</i> | <i>F. nucleatum</i> | <i>P. gingivalis</i> | <i>P. intermedia</i> | <i>A. actinomycetemcomitans</i> |
|----------------------|---|--------------------|--------------------------|---------------------|---------------------|----------------------|----------------------|---------------------------------|
| Erythromycin | R | 21(60%) | 28(31%) | 14(42%) | 4(33%) | 1(17%) | 2(50%) | 2(50%) |
| | S | 12(34.%) | 56(62%) | 17(52%) | 8(67%) | 5(83%) | 2(50%) | 1(25%) |
| | I | 2(6%) | 6(7%) | 2(6%) | 0 | 0 | 0 | 1(25%) |
| Tetracycline | R | 6(17.1%) | 4(4%) | 10(30%) | 3(25%) | 2(33%) | 2(50%) | 1(25%) |
| | S | 29(82.9%) | 86(96%) | 23(70%) | 9(75%) | 4(67%) | 2(50%) | 3(75%) |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amoxicillin | R | 3(8.6%) | 3(3%) | 8(24%) | 2(16%) | 1(17%) | 1(25%) | 0 |
| | S | 32(91.4%) | 87(97%) | 25(76%) | 10(84%) | 5(83%) | 3(75%) | 4(100%) |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amox./clavul. | R | 2(5.7%) | 3(3%) | 8(24%) | 2(16%) | 1(17%) | 1(25%) | 0 |
| | S | 33(94.3%) | 87(97%) | 25(76%) | 10(84%) | 5(83%) | 3(75%) | 4(100%) |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Metronidazole | R | 24(68.6%) | 18(20%) | 31(94%) | 9(75%) | 4(67%) | 3(75%) | 4(100%) |
| | S | 11(31.4%) | 72(80%) | 2(6%) | 3(25%) | 2(33%) | 1(25%) | 0 |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clindamycin | R | 15(42.9%) | 11(12%) | 7(21%) | 1(8%) | 1(17%) | 1(25%) | 1(25%) |
| | S | 20(57.1%) | 79(88%) | 26(79%) | 11(92%) | 5(83%) | 3(75%) | 3(75%) |
| | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Azithromycin | R | 23(65.8%) | 16(18%) | 13(39%) | 5(42%) | 2(33%) | 1(25%) | 1(25%) |
| | S | 6(17.1%) | 70(78%) | 19(58%) | 7(58%) | 4(67%) | 3(75%) | 2(50%) |
| | I | 6(17.1%) | 4(4%) | 1(3%) | 0 | 0 | 0 | 1(25%) |
| Linezolid | R | 1(2.9%) | 0 | 1(3%) | 1(8%) | 0 | 0 | 1(25%) |
| | S | 32(91.4%) | 90(100%) | 31(94%) | 10(84%) | 6(100%) | 3(75%) | 2(50%) |
| | I | 2(5.7%) | 0 | 1(3%) | 1(8%) | 0 | 1(25%) | 1(25%) |

followed by gramnegative strict anaerobes (*Fusobacterium* and *Prevotella*)(14-16).

As regards antibiotic susceptibility, Tanner et al. (6) in a study of dentoalveolar abscesses reported facultative anaerobes to exhibit similar percentages of resistance to amoxicillin and amoxicillin / clavulanate (7%). This figure was in turn seen to increase in the case of strict anaerobes to 13%. Gilmore et al. (17) in turn reported strict anaerobe resistance to penicillins to range from 8.9-16%, depending on the genus involved. In our study, for facultative anaerobic bacteria, the resistances were slightly lower (4.8% and 4.0%, respectively), while for strict anaerobes, our documented resistance values were higher (21.8%)(Table 2).

Other authors have reported high anaerobe resistance to penicillins (18-22), since the patients involved had severe conditions and had been previously and ineffectively treated with antimicrobials on an outpatient basis.

Unlike Gilmore et al. (17) and Levison et al. (23), who found low percentages of resistance to clindamycin in anaerobic bacteria, we recorded relatively high resistance in terms of absolute values (19.6%)(Table 2).

Herrera et al. (24), in a comparative study of periodontal abscess treatment with amoxicillin / clavulanate versus azithromycin, found both treatment modalities to be equally effective. Ingham et al. (25) reported the efficacy of metronidazole and penicillins to be similar in the treatment of odontogenic infections. Similar observations were published by Khemaleelakul et al. (26),

who in their comparative study of penicillin V, metronidazole, amoxicillin, amoxicillin / clavulanate and clindamycin for the treatment of cellulites of periapical origin, found no significant differences among the treatments. In our study we detected an alarming increase in resistance to metronidazole.

Baumgartner et al. (27), in their comparative study of penicillin V, amoxicillin, amoxicillin / clavulanate, clindamycin and metronidazole for the treatment of abscesses of periapical origin, only observed resistance to metronidazole – in coincidence with our own results.

A high rate of resistance to azithromycin has also been seen as compared to amoxicillin and amoxicillin / clavulanate. A possible explanation for this is that broad-spectrum antibiotics are routinely used in our setting to combat mild infections, and use is very specifically made of those drugs causing the destruction of gramnegative anaerobes (28-30). Such over-treatment affects the resistances of both the oral flora and of other ecosystems.

The results of our study allow us to draw the following conclusions relating to clinical practice and antibiotic prescription:

1.- Odontogenic infection, both of periapical origin and caused by third molar pericoronitis, is most often produced by anaerobic bacteria.

2.- The antibiotic susceptibility of these bacteria is very high in the case of amoxicillin, amoxicillin / clavulanate, linezolid, tetracycline and clindamycin – regardless of the origin of the odontogenic infection.

3.- Although we have recorded modest resistance to amoxicillin in infections of periapical origin or associated to pericoronaritis, we are of the opinion that this drug remains the treatment of choice for infections of this kind. The presence of clavulanic acid does not constitute a decisive advantage in the management of these patients.

4.- Clindamycin should be the alternative treatment choice in the event of amoxicillin or amoxicillin / clavulanate failure, as well as in patients who are allergic to penicillin.

5.- A number of antibiotic substances considered to date to be effective in treating odontogenic infections, such as metronidazole, erythromycin and azithromycin, show a high proportion of resistances.

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