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TITLE PAGE

ARE ROUTINE MICROBIOLOGICAL SAMPLINGS IN ACUTE DENTAL INFECTIONS
JUSTIFIED? OUR 10 YEAR'S REAL-LIFE EXPERIENCE.

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Concise title: MICROBIOLOGICAL SAMPLING IN ODONTOGENIC INFECTIONS.

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ARE ROUTINE MICROBIOLOGICAL SAMPLINGS IN ACUTE DENTAL INFECTIONS JUSTIFIED? OUR 10-YEAR REAL-LIFE EXPERIENCE

ABSTRACT

Purpose:

Most patients with severe odontogenic infections are successfully treated with large spectrum probabilistic antibiotic therapy, drainage of the collections and tooth treatment or extraction and are discharged home before antibiotic sensitivity results were available. The investigators hypothesized whether bacteriological sampling should be systematically performed in the management of patients with severe odontogenic infections.

Methods: The investigators implemented a prospective observational study. The sample was composed of patients managed between January 2004 and December 2014 for severe odontogenic infection based on three criteria: hospital admission, intravenous antibiotic therapy, tooth extraction and collections drainage under general anesthesia. The predictor variable was the results of bacteriological sampling, culture and sensitivity. The outcome variable was antibiotic therapy adaptation according to antibiotic sensitivity results.

Results: The sample was composed of 653 patients; 386 (59%) were male and 267 (41%) female, with a mean age of 37 years (range 18 – 88); 378 (58%) patients had been receiving oral antibiotics before admission to hospital, for a mean duration of 4.1 days (range 1 – 30). About 535 (81.9%) patients had swabs taken during surgery. Microorganisms were observed in 477 (89.1%) patients but in 377 (70.5%) they were polymorphic oropharyngeal flora. After culture, at least one antibiogram was obtained for 91 (17%) patients and the results led to antibiotic therapy being adapted in 23 (4.3%) patients.

Conclusion:

The results suggest that bacteriological analysis had an impact on evolution in less than 5% of

patients. Future studies will focus on the patients for whom the bacteriological analysis is essential.

KEYWORDS: Odontogenic; infections; antibiotics; microbiology; sensitivity test.

INTRODUCTION

With the advent of antibiotics such as penicillin and better dental care, odontogenic infection is now considered to be an easily treatable condition. However, despite the improvement in living standards, health and quality of life, odontogenic infections are still a common occurrence. North American and European studies report increasing rates of emergency department visits and hospitalizations for acute dental infections[1]. Severe odontogenic infection is a disease entity with potentially systemic complications. Treatment guidelines are surgical incision and drainage of the purulent collections in combination with extraction of the tooth concerned or root canal treatment, oral cavity rehabilitation, probabilistic parenteral antibiotic therapy and hospitalization². In France, non-severe odontogenic infections are managed by the dentist while hospitals deal with all severe cases involving trismus, extensive cellulitis, dysphagia, dysphonia, dyspnoea and refractory pain. Various reports state that odontogenic infection is due to the interdependent and synergistic metabolism of a variety of mixed aerobic and anaerobic microorganisms whose collection and culture are complex[2–5,5–12,12,13,13–15]. Empirically and in our everyday experience, we have observed that the probabilistic antibiotic therapy is rarely modified during a hospital stay. The objective of this prospective study was to determine if bacteriological sampling provides relevant information for the management of patients with acute dental infections and whether this procedure could be reserved for a specific population.

MATERIAL AND METHODS

Recruitment

We performed an ancillary observational study prospectively between January 2004 and December 2014 in the Department of Oral and Maxillofacial Surgery, CHU Clermont-

Ferrand, France, in accordance with the ethical principles of the World Medical Association Declaration of Helsinki for medical research involving human subjects (CE-CIC-GREN-12-08).

All patients enrolled in the study had an acute dental infection as defined by the following criteria: hospital admission, intravenous antibiotic treatment and tooth extraction with incision and drainage under general anaesthesia. Patients who could be managed under local anaesthesia were directly referred to the Department of Odontology.

Data collection

The following data were collected:

- socio-demographic data: age, sex, associated medical conditions such as penicillin allergy, smoking status, alcohol consumption, psychiatric disorder, immunodepression [16] (diabetes mellitus, obesity [17], chemotherapy, HIV and hepatitis B or C infection, transplant surgery), asthma, non-mellitus diabetes
- number and type of spaces involved by the infection
- treatment on admission: ongoing antibiotic and/or anti-inflammatory treatment
- duration of hospitalization and number of surgical procedures
- bacteriological results, results of culture and sensitivity testing
- antibiotic adaptation

Patient management

On admission, a medical history was taken and results of clinical examination, C-reactive protein (CRP) assay and white blood count were recorded. The biological results provided no relevant information and hence are not given in the text. Dental panoramic radiography was systematically performed and in case of symptoms such as dyspnoea or dysphagia a

cervicofacial CT-scan. After the airway had been secured, the causal tooth was extracted under general anaesthesia, with incision and drainage via the intraoral and/or extraoral sites. Debridement was performed of all cervical facial spaces involved by infection. All patients received intravenous probabilistic antibiotic treatment against oral mucosal flora. The French Health Products Safety Agency (ANSM) recommends amoxicillin and clavulanic acid (1 gram every 8 hours), and clindamycin (300 mg every 8 hours) and metronidazole (500 mg every 8 hours) in case of penicillin allergy. If necessary, antibiotics were adapted according to bacteriological results (Recommendations of the French Health Products Safety Agency, 2003).

Microbiological culture and sensitivity

Pus was sampled in patients at the first surgical procedure and in the event of repeat surgeries. Bacteriological diagnosis began with direct examination, after which the swab was fixed and Gram-stained. Culture was then performed under aerobic and anaerobic conditions. Samples were seeded on Columbia blood agar plus colistin and nalidixic acid (CNA), and on chocolate agar plus PolyViteX (bioMérieux, France) incubated at 37 °C in CO₂-enriched atmosphere at 5%. The dishes were checked daily for 3 days. Anaerobic bacteria and anaerobic agar (Oxoid, France) were seeded and incubated at 37 °C using jars and packets generating an anaerobic atmosphere (Anaerogen Oxoid, France) for at least 5 days. Dishes were checked daily after 48 hours of culture. The bacterial species were identified with the API 20 A (bioMérieux) and Rapid ID 32A test kits.

The cost of microbiological culture and sensitivity testing of an abscess sample in France is 54 euros.

Statistical analysis

Descriptive statistics are expressed as mean and range (min-max), according to their distribution, and number of patients (%) for categorical data. Statistics were computed with STATA V12 (Stata Corp, College Station, Texas, USA). Tests were two-sided and a p-value<0.05 was considered statistically significant.

RESULTS

Data collection

During the period of the study, 653 patients were enrolled: 386 (59%) were male and 267 (41%) female, with a mean age of 37 years (range 8 – 88), 375 (57%) were smokers, 78 (12%) regular drinkers, 54 (8%) addicted to drugs, 23 (3.5%) non-mellitus diabetics, 25 (3.8%) immunosuppressed, 33 (5%) asthmatics and 62 (9.5%) had psychiatric disorders. Forty seven patients (7.2%) were allergic to penicillin. During surgery, 516 patients (80%) had a single facial space infected by pus, 95 (15%) patients 2 spaces, 28 (4%) patients 3 spaces, 5 patients (1%) 4 spaces and 1 patient had 6 spaces involved. The submandibular space 32% (n=209) and the vestibular space 28% (n=182) were the most frequently involved.

A total of 378 (58%) patients had been receiving oral antibiotics before admission to hospital, for a mean duration of 4.1 days (range 1 – 30). Most patients (231: 61%) had been prescribed amoxicillin or amoxicillin and clavulanic acid, 75 (20%) had a combination of spiramycin and metronidazole, 37 (10%) pristinamycin, 36 (9.5%) metronidazole and 2 (0.5%) clindamycin.

Microbiological culture and sensitivity testing (Fig.1)

A total of 535 (81.9%) patients had swabs taken during surgery, 118 (18.1%) were excluded from the study because bacteriological sampling was not performed. It is noteworthy that there was no adverse clinical outcome for any of these 118 patients. In 58 patients (10.9%)

Gram-staining identified no microorganism. In 100 of the 477 (89.1%) patients with positive examination, one to three non-commensal or pathogenic bacterial species were isolated. In the remaining 377 no predominant bacteria were isolated (polymorphic oropharyngeal flora). Antibiotic sensitivity testing was performed in the group of 100 patients (18.7%) with one to three bacteria isolated on culture. In 77 patients, the probabilistic antibiotic initially prescribed was active against the bacteria isolated. Antibiogram results led to antibiotic therapy adaptation in the remained 23 patients, of whom 10 were discharged because of favourable clinical evolution before results were available. About 20% of patients under antibiotic therapy before admission had a germ identification and 17% of patients without any antibiotic treatment before sampling, with no significant statistical difference.

Analysis of swab samples showed that 107 (55.7%) patients had aerobic flora, 79 (37.7%) anaerobic flora and 14 (6.6%) mixed aerobic-anaerobic flora. Gram-positive cocci were isolated in 103 (19.2%) cultures. The most common bacteria isolated were group F *Streptococcus* (22 swabs), α -hemolytic *Streptococcus* (18 swabs), and *Streptococcus constellatus* (16 swabs). (Tab. 1). Among the antibiograms performed, 23% contained bacteria resistant or with intermediate sensitivity to amoxicillin-clavulanic acid, 78% resistant to gentamycin, 36% to clindamycin and 2% to pristinamycin.

Forty-two patients (6.4%) had more than one surgical procedure to drain collections. All had undergone new sampling during second-look surgery. The mean duration of their hospital stay was 17.3 days (range 5-37 days). Five of them (12%) were immunocompromised and therefore had a statistically significant greater risk of developing complications than non-immunocompromised patients ($p= 0.001$); 13 (30.9%) patients needed antibiotic adaptation subsequent to the first or other samplings.

DISCUSSION

Our study showed that 83% of patients had no probative microbiological results, either because there was no germ growth or because polymorphic oropharyngeal flora was present. Culture and sensitivity testing was possible for only 18.6% patients. Antibigram results led to antibiotic adaptation in 23 patients (4.3%). In only 13 patients (2.4%) did it improve clinical symptoms, all of them were in the group of patient with several surgeries et complex evolution..

Clinicians are aware of the difficulties in taking samples for qualitative microbiological testing[19]. In our study, patient management was always performed in the emergency department by different surgeons, of whom some had forgotten to perform sampling and others thought that it was unnecessary. Two different sampling techniques exist, swabbing and aspiration. Aspiration is more effective: it recovers more anaerobes than swabbing [20] and decreases the risk of contamination from the skin, oral mucosa and saliva. Bacteria and more specifically anaerobes are sensitive to milieu change in the time between sampling and transport to the laboratory [2,20]. Anaerobic strains need more time in culture to grow [21]. Hence, isolating and identifying a bacterium are difficult and the isolated microorganism may not be representative of the pathogenic bacteria.

Moreover, in the study, 58% of patients had antibiotic therapy before their hospitalisation in our Department, which could prevent or delay bacteria identification. When culture enabled us to isolate bacteria, the most commonly observed in the last four decades, as in other studies, was *Streptococcus alpha-haemolyticus* [7,8,15,21,22] and more specifically, *Streptococcus Viridans*[3]. Obviously, probabilistic antibiotic therapy must target it. Our results show that 23% of the bacteria were resistant to combined amoxicillin and clavulanic acid and 36% to clindamycin. In the literature, the rate of susceptibility to penicillin varies from 100% [20,23] to 70% [3,8]. In the study of Farmahan et al., resistance to amoxicillin was

26.6%, and 18.7% to a combination of amoxicillin and metronidazole [3]. The incidence of resistance to antibiotics routinely used in deep space head and neck infection is 18% for clindamycin, 14% for macrolides and 7% for penicillin G in the aerobic spectrum, and 11% for clindamycin, 6% for metronidazole and 8% for penicillin G in the anaerobes [24]. However, in the study of Poeschl et al.[24] no clinical antibiotic failure was observed in patients treated by an association of amoxicillin and clavulanic acid. Warnke et al.[25] found that amoxicillin with clavulanic acid would have been sufficient as a single agent in only one third of the patients. However, these *in vitro* results do not reflect clinical success because in the study an absolute majority of patients treated with penicillin and abscess drainage had a rapid improvement in clinical symptoms [23]. The clinical efficacy of penicillin could be the result of the vulnerability of the dominant strains to the antibiotic [3,8,25], coupled to collection drainage. The relation between probabilistic antibiotic treatment and bacterial resistance is unclear.

At least 24 to 48 hours are required to isolate a bacterium, a period that can be extended by 48 hours to isolate a dominant bacterium when there are polymorphic flora or in the event of antibiotic treatment before sampling (which was the case for 58% of patients in our study). About 4 to 6 days are needed to obtain an antibiogram if a bacterium is isolated. In our study, 493 (92%) patients were discharged after 5.4 days on average and successfully treated before the results of culture and sensitivity testing were available.

Based on our findings, 83% of patients had no probative microbiological results and 92% are discharged home before results are available; this suggest that bacteriological analysis had no impact on their evolution.

In light of these results and those from the literature, pus sampling in the management of patients with severe odontogenic infection could be restricted to a well-defined type of patient.

However, immunocompromised patients have a higher risk of needing repeat surgery and of developing systemic infection and cervical necrotizing fasciitis [7,11], we think that for those patients systematic swabs is essential. Also, patients with unfavourable evolution and/or admitted to an intensive care unit (ICU) and/or with multiple drainage, samplings are necessary during a second look. Complementary studies should be performed to identify patients with predictive factors of complications for whom systematically sampling may be necessary (Fig.2).

Compliance with Ethical Standards

Conflict of Interest:

Author Nathalie Pham Dang declares that she has no conflict of interest.

Author Candice Delbet-Dupas declares that she has no conflict of interest.

Author Arnaud Depeyre declares that he has no conflict of interest.

Author Aurélien Mulliez declares that he has no conflict of interest.

Author Laurent Devoize declares that he has no conflict of interest.

Author Isabelle Barthélémy declares that she has no conflict of interest.

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Ethical approval:

This article does contain studies with human participants.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:

For this type of study, formal consent is required.

Informed consent was obtained from all individual participants included in the study.

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FIGURE CAPTATION

Figure 1: **From sampling to clinical relevance.**

On the right side are reported the number of patients for whom a bacteriological analysis was performed including sampling, direct observation, culture and sensibility and finally the clinical relevance. The light grey coloration correspond to patients with a positive result. The percentage are calculated by reference to the 535 for whom a sampling was performed.

Figure 2: **Decisional tree**

Proposition of decisional tree face to patient with severe odontogenic infection.

Inclusion

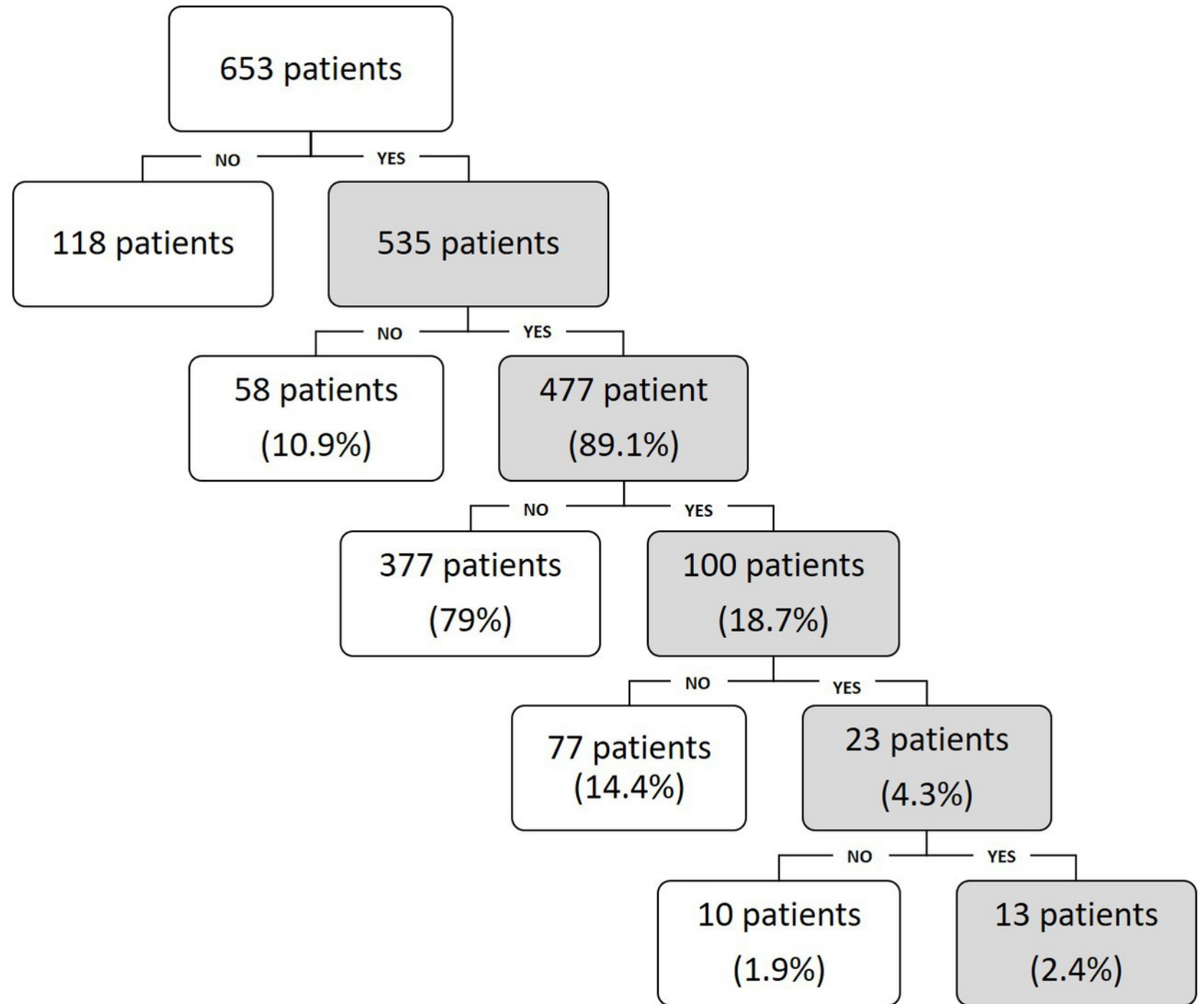
1st step: sampling performance

2nd step: Gram staining allow to pathogenic bacterial species identification

3rd step: microbiological culture allow to microbial organism proliferation

4th step: antibiotic susceptibility testing allow to identify inadequation between microbial organism sensitivity and antibiotic therapy

5th step: culture and sensitivity impact patient recovery



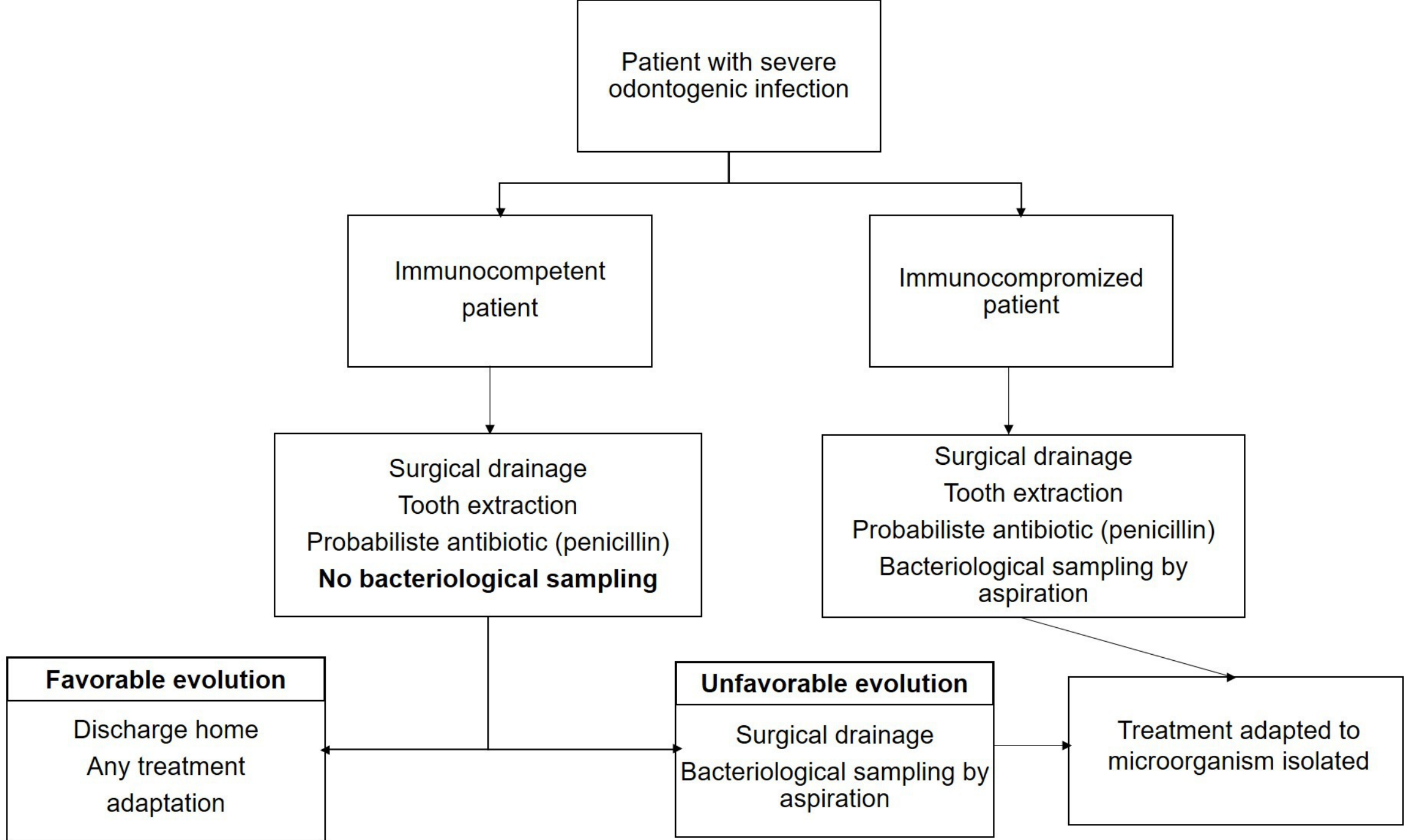


Table 1: Microorganisms identification among bacteriological sampling from 535 patients.

Microorganisms	Number	Microorganisms	Number
OROPHARYNGEAL FLORA	377 (70.5%)		
AEROBES	132 (24.6%)	ANAEROBES	75 (21.2%)
Gram-positive Cocci	103 (19.2%)	Gram-positive	4 (0.7%)
Staphylococcus		<i>Actinomyces</i>	3
<i>S. aureus</i>	7	<i>Peptostreptococcus</i>	1
<i>S. coagulase negative</i>	4		
<i>S. epidemidis</i>	4	Gram-negative	7 (1.3%)
Undetermined	2	<i>Prevotella</i>	4
Streptococcus		<i>Capnocytophaga</i>	2
Groupe A		<i>Eikenella</i>	1
Alpha-hemolytic	18		
Beta-hemolytic	3	YEAST STRAIN	30 (5.6%)
Group C	3	<i>Candida albicans</i>	23
Group F	22	<i>Malassezia</i>	1
Group G	7	<i>Geotrichum</i>	1
Complex Milleri		Undetermined	5
<i>Str. anginosus</i>	7		
<i>Str. constellatus</i>	16		
<i>Str. intermedius</i>	2		
Undetermined	5		
Enterococcus			
<i>E. faecium</i>	1		
Undetermined	2		
Gram-positive Bacillus	3 (0.5%)		
<i>Corynebacterium</i>	3		
Gram-negative Bacillus	26 (4%)		
Enterobacter			
<i>Citrobacter</i>	1		
<i>E. cloacae</i>	3		
<i>E. coli</i>	2		
<i>Klebsiella</i>	3		
<i>M. morganii</i>	2		
<i>Proteus</i>	4		
<i>Serratia spp</i>	1		
Undetermined	2		
Coccobacillus			
<i>Haemophilus</i>	3		
<i>Acinetobacter</i>	2		
<i>P. aeruginosa</i>	3		