

Evaluation of Antibiotic Resistance of Cariogenic Isolates from Korhogo University Hospital Center from July to September 2024

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Abstract

This work consisted in determining the resistance profile of bacterial isolates from dental caries, from the dental office of the GON COULIBALY Peleforo University of Korhogo. The samples taken were preserved in deep agar and the identification was done using the MALDI TOF technique. The sensitivity of the identified strains was carried out according to the technique of the Antibio-gram Committee of the French Society of Microbiology (CA SFM, 2024). The 25 samples revealed 25 identified strains divided into 5 families including the Pseudomonaceae family with 20% frequency, the Staphylococcaceae family (16%), the Enterobacteriaceae family (56%), the Enterococcaceae family (4%) and the Sterotrophomonaceae family (4%). The antibiogram carried out revealed total resistance of pseudomonas and Enterobacteriaceae to Piperacillin tazobactam (TZP). As for Amoxicillin plus clavulanic acid (AMC), resistance was also total for Enterobacteriaceae. However, Staphylococcaceae showed total resistance to Tetracycline (TET) and Tobramycin (TOB). The other antibiotics levofloxacin (LVX), ceftazidime (CAZ), piperacillin (PIR) showed intermediate sensitivity to almost all strains tested. It is noted that the antibiotic profile of the strains studied varies according to the family and that the multi-resistance of almost all of the different strains identified is proven, which consequently would be a monitoring indicator for our clinical prescribers and others (national and international decision makers on the use of antibiotics).

Keywords

Bacterial Isolates, Resistance, Carious Teeth, Antibiotics, Maldi ToF

1. Introduction

Caries pathology is a microbial condition, mainly bacterial, which nowadays is causing gangrene in our populations. Ranked 4th globally, it comes after cancer, diabetes and AIDS (Acquired Immuno-Deficiency Syndrome) [1]. In fact, 80% of the world population, 60% to 90% of whom are school-aged children, and 100% adults suffer from this disease. In Ivory Coast this condition rate is 60% and constitutes a public health problem [2].

Antibiotics are natural substances (produced by microorganisms) or synthetic (Laboratory), with antimicrobial activity relatively at low concentrations. Their property is to eliminate or inhibit the growth of microorganisms [3]. However, in oral medicine, antibiotics remain particularly useful, because the treatment of an infectious source is most often non-drug. Antibiotics are useful when they can help the host defenses to eliminate residual microorganisms not destroyed by appropriate local treatment [3]. Despite the instructions for the use of antibiotics in endodontics, prescriptions are only increasing. Thus, Beta lactams are sufficiently prescribed with 65.2% consumption [4]. Also, it is remarkable that the prescriptions are not adapted [5], which consequently indicates an insufficiency in antibiotic prophylaxis and in the choice of antibiotic therapy [6]. Furthermore, in Ivory Coast, street drugs, which are widely used, represent potential health hazards. Indeed, less than 20% of national pharmaceutical coverage for the sale of drugs is available. This situation, which undoubtedly remains a key factor in the emergence of bacterial resistance, it seemed important to us to evaluate the resistance of caries strains to current antibiotics. The objective of this work is to determine the resistance profile of bacterial isolates from carious teeth from the dental office of the GON COULIBALY Péléforo University.

2. Materials and Methods

This work took place during the period from July 7 to September 30, 2024. The samples of carious teeth were taken at the dental office of Péléforo GON COULIBALY University. The identification and antibiogram were carried out at the Institut Pasteur de Cote d'Ivoire (IPCI).

2.1. Materials

Caries teeth were extracted from patients whose treatment requires tooth extraction. Patient ages range from 10 to over 50 and of all genders. Nutrient and ordinary agar as well as Muller Hinton culture medium were used. The MALDI TOF device and antibiotic blotting discs were also used. Finally, all the technical equipment of the Microbiology Laboratory was used.

2.2. Methods

2.2.1. Extraction and Conservation of Carious Teeth

After consulting the patient, the dentist assesses whether there is a need to extract the diseased tooth or not. If yes, then the tooth is extracted with all the Kit available to the dentist. Once extracted, the carious tooth is preserved in deep agar in Eppendorf tubes prepared for this purpose, then stored at office laboratory temperature.

2.2.2. Identification Using MALDI TOF

The bacterial sample called the calibrating strain (*E. coli*) is placed on the plate beforehand. Using a platinum loop, an isolated colony is taken and deposited on the microplates in the form of a smear which corresponds to the positive control (T+). In the same column and in the second line, a second spot called negative control (T-) is placed and each spot is covered with 1 μ L of saturated matrix α -cyanohydroxamic acid (ACHC). This operation is repeated for each colony to be identified. Then the MALDI TOF target is left to dry on the bench for 15 min. The plates are scanned on the computer and this step constitutes the information phase of the preparation station (PRE-STAT). Thus, the information concerning each colony isolated on the plate are represented on the computer. A second computer constituting the Sample Acquisition or Analysis Monitoring Station (ACQ-STAT) allows the MALDI TOF device to be launched by command. The plate carrier is removed, and the microplates are inserted inside. Each plate is scanned in order to record the information on the second computer. Once this operation is completed, the device closes automatically. After a few hours of analysis, the MS (Mass Spectrometer) software displays the results in the "MYLATM" program and indicates the percentage of identification by measuring the very abundant proteins in the bacteria [7]. Indeed, the characteristic patterns of these very abundant proteins have been used to reliably and very precisely identify bacterial strains. Thus, the results of a target are validated if and only if (T+) corresponds to *E. coli* at 99.9% and (T-) corresponds to a score without identification lower than 99.99%.

2.2.3. Sensitivity of Identified Strains to Antibiotics

To determine the resistance profile of the different strains isolated, a standardized antibiogram in agar media in several stages is carried out according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [8].

1) Preparation of the bacterial inoculum

From a young culture of 18 to 24 hours grown on ordinary agar, a bacterial suspension is prepared. A pure bacterial colony is taken using a swab and suspended in a hemolysis tube containing 3 mL of 0.85% NaCl saline solution or physiological water (Api[®] Biomérieur[®]). The suspension is then homogenized using a vortex. The density of the mixture is adjusted using a Densicheck[®] to obtain 0.5 Mc Ferland or 5.105 CFU/mL [8]. The inoculum can be adjusted by adding

either a bacterial culture if it is weak, or physiological water (NaCl) if it is strong. Inoculation is done after 15 minutes following preparation of the inoculum.

2) Seeding on Mueller-Hinton (MH) agar for pseudomonas

1 μ l of the bacterial inoculum is introduced into a hemolysis tube containing 3 ml of saline solution (NaCl), then homogenized well using a vortex and finally inoculated over the entire surface of the Mueller-Hinton agar in tight streaks, rotating the petri dish each time [8].

3) Seeding on MH for staphylococcus

1000 μ l of the bacterial inoculum are introduced into a hemolysis tube containing 3 mL of physiological water then homogenized using a vortex and finally inoculated over the entire surface of the Mueller-Hinton agar in tight streaks, rotating the petri dish each time [8].

4) Seeding on MH for Enterobacteria and other families

100 μ l of the bacterial inoculum is introduced into a hemolysis tube containing 3 mL of physiological water and homogenized well and finally inoculated on the entire surface of the Muller-Hinton agar in tight streaks, rotating the petri dish each time [8].

5) Application of antibiotic discs

The antibiotic disks are brought to room temperature, then using a manual applicator placed on the agar 30 mm from each other and from the edge of the petri dish. After application of the discs, the seeded petri dishes are left at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) on the bench for approximately 15 minutes to allow pre-diffusion of the antibiotic discs. Incubation takes place at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$, aerobically, for 20 ± 4 h [8].

6) Interpretation of results

The inhibition diameters are measured using a caliper and the inhibition values are obtained in (mm). Using the different classes of strains: (S) for sensitive ones, (I) for intermediate ones and (R) for resistant ones, in accordance with the standards [8], the distribution is thus made.

3. Results

During the study period, a total of 56 patients were consulted with 25 dental extractions. The age of people who have undergone tooth extraction ranges from 08 to 60 years. The different extraction rates depending on age are given as shown in the diagram below (Figure 1).

The distribution of patients who had at least one tooth extracted during the survey period according to sex or gender is represented by the diagram below with 60% female and 40% male (Figure 2).

As a result of the identification by the MALDI-TOF method the MALDI-TOF method made it possible to identify 25 species of different strains corresponding to the 25 samples taken, with an accuracy of 99.9% of almost all the strains analyzed except 5 strains which displayed a purity of 50% (Table 1).

Analysis of the isolates from the samples using MALDI TOF revealed 25 different

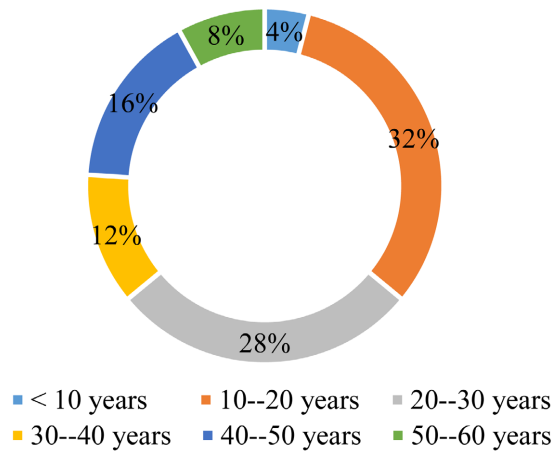


Figure 1. Diagram representing the rate of extraction of carious teeth as a function of the age of the patients.

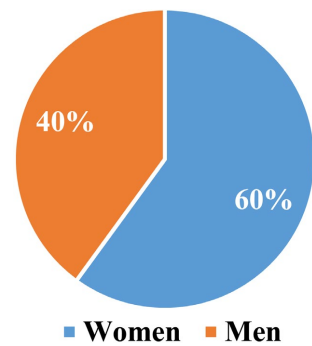


Figure 2. Diagram representing the rate of gender of patients having extracted at least one tooth during the survey period.

Table 1. Table representing the nature of the strain contained in different isolates, identification by MALDI TOF from the UPGC dental office.

Order number	Strain number	Strain name	Confidence level (%)
1	P1KO-1	<i>Klebsiella pneumoniae</i>	99.9
2	P2KO-1	<i>Citrobacter koseri</i>	99.9
3	P11KO-1	<i>Pluralibacter gergoviae</i>	99.9
4	P8KO-1	<i>Klebsiella pneumoniae</i>	99.9
5	P9KO-1	<i>Pseudomonas stutzeri</i>	99.9
6	P10KO-1	<i>Pseudomonas putida</i>	99.5
7	P19KO-1	<i>Enterococcus faecalis</i>	99.9
8	P17KO-1	<i>Staphylococcus epidermidis</i>	99.9
9	P14KO-1	<i>Stenotrophomonas maltophilia</i>	99.9
10	P15KO-1	<i>Pseudomonas aeruginosa</i>	99.9
11	P16KO-1	<i>Pseudomonas aeruginosa</i>	99.9
12	P12KO-1	<i>Staphylococcus saprophyticus</i>	99.9

Continued

13	P13KO-1	<i>Klebsiella pneumoniae</i>	99.9
14	P20KO-1	<i>Klebsiella pneumoniae</i>	99.9
15	P23KO-1	<i>Klebsiella pneumoniae</i>	99.9
16	P25KO-1	<i>Pseudomonas aeruginosa</i>	99.9
17	P3KO-1	<i>Enterobacter hormaechei</i>	83.1
18	P7KO61	<i>Staphylococcus saprophyticus</i>	99.9
19	P4KO-1	<i>Staphylococcus arlettae</i>	99.9
20	P6KO6-1	<i>Klebsiella pneumoniae</i>	92.8
21	P18KO-1	<i>Enterobacter cloacae</i>	50.0
22	P22KO-1	<i>Enterobacter asburia</i>	50.0
23	P21KO-1	<i>Enterobacter cloacae</i>	50.0
24	P24KO-1	<i>Enterobacter asburiae</i>	50.0
25	P5KO-1	<i>Enterobacter asburiae</i>	50.0

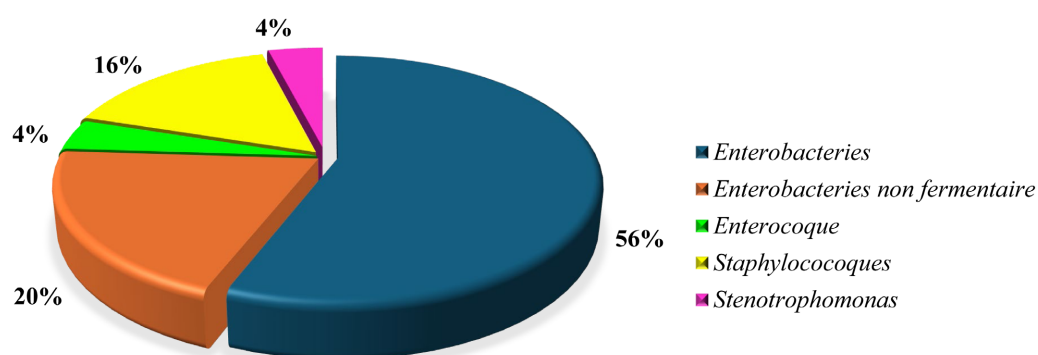


Figure 3. Diagram representing the dominance rate of the families of the isolated strains.

strains divided into 5 families as shown in the diagram below (**Figure 3**). Only the Enterobacteriaceae family was the majority (56%) of the different families represented.

Antibiogram of the different strains isolated from the carious teeth of the patients. The different strains isolated from the carious teeth were the subject of a sensitivity study to the different antibiotic disks. Thus, for the *Staphylococcus strains* 7 antibiotic disks were used on 04 strains from patients P4, P7, P12, and P17. It emerges from this analysis that the *Staphylococcus strains* are resistant to tetracycline (TET) with inhibition diameters less than 18 mm. The same is true for tobramycin (TOB) except for the strain from patient P7 which is sensitive to (TOB) with a diameter equal to 28 greater than 18. As for the antibiotics levofloxacin (LVX) and Ciprofloxacin (CIP), they remain of intermediate sensitivity to all strains of *Staphylococcus* studied (**Table 2**).

The sensitivity of *Pseudomonas strains* required 06 antibiotic disks for 05 strains from patients P9, P10, P15, P16 and P25. It appears from this analysis that

all the strains subjected to the study showed resistance to piperacillin-tazobactam (TZP) with inhibition diameters less than 20 mm. However, Ceftazidime (CAZ) and piperacillin (PIR) were of intermediate sensitivity to contact with all *Pseudomonas strains* (Table 3).

Table 2. antibiogram of the isolated *Staphylococcus strains*.

ATB Patients	TET	ERY	TOB	CN	FOX	LVX	CIP
P4	06	06	16	14	28	22	28
P7	16	32	28	28	28	22	28
P12	06	06	12	14	20	22	22
P17	06	28	06	22	24	22	28

Sensitive (S), resistance (R), Intermediate (I). Erythromycine (ERY), Tobramycine (TOB), Gentamycine (CN), Lévofoxacine (LVX), Ciprofloxacine (CIP), Céfoxitine (FOX), Tétracycline (TET).

Table 3. Antibiogram of isolated *Pseudomonas strains*.

ATB Patients	TZP	LVX	MEM	AK	CAZ	PIR
P9	06	28	23	17	20	20
P10	06	20	25	18	22	22
P15	06	27	35	19	22	24
P16	06	24	28	22	23	22
P25	06	23	33	20	23	20

Concerning Enterobacteria, out of 14 antibiotic disks submitted to the study, only 02 antibiotics (Amoxicillin plus clavulanic acid (AMC) and piperacillin-tazobactam (TZP)) were subject to absolute resistance to the 15 strains tested with inhibition diameters lower than 16 and 20 mm respectively (Table 4). But as for piperacillin (PIP) and fosfomycin (FOS), they were of relative sensitivity. The other antibiotics except these 04 mentioned, were almost sensitive to all the strains studied as indicated in the table below with diameters greater than 22 mm.

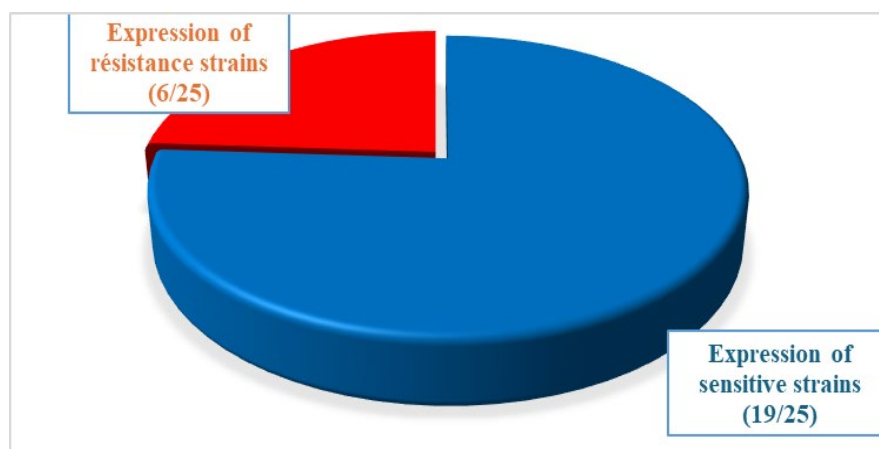
Of the 25 strains studied, 06 strains show resistance expression, *i.e.* 24%, and 19 strains show sensitivity expression, *i.e.* 76% (Figure 4).

4. Discussion

The prospective study of the resistance of cariogenic bacteria allowed us to identify 25 distinct strains distributed according to age, sex and by family of bacteria. Thus, the patients with carious teeth from which the strains came were those whose ages ranged from 10 to 20 years and from 21 to 30 years. This result is like those of [9], who determined during their work on the evaluation of antibiotic prescriptions for patients suffering from dental pain, an age group of 25 to 34 years more represented. This representation could be explained by the fact that it

Table 4. Antibiogram of isolated enterobacteria strains.

ATB Patients	AMC	LVX	AK	PIP	FOX	CRO	MEM	FOS	TZP	PEF	GMN	ETP	CTX	CZD
P1	06	28	17	06	22	07	27	18	06	25	21	28	06	08
P2	06	32	22	16	23	30	27	27	06	29	21	32	24	27
P3	06	26	17	14	22	27	24	17	06	23	19	10	22	23
P5	06	39	25	26	35	30	22	27	06	26	21	36	24	30
P6	06	27	20	16	19	26	25	15	06	24	19	29	28	24
P8	06	18	20	15	24	30	26	20	06	13	19	30	29	24
P11	06	30	22	20	35	30	25	20	06	29	22	35	22	31
P13	06	29	24	24	29	28	25	22	06	25	20	30	23	23
P18	06	29	25	25	29	27	24	15	06	23	20	29	22	22
P20	06	24	20	18	06	26	26	16	06	21	18	27	22	24
P21	06	37	22	27	30	25	19	15	06	32	30	34	31	32
P22	06	37	22	27	30	25	19	16	06	30	29	33	29	30
P23	06	38	22	20	28	28	26	20	06	25	20	29	24	25
P24	06	37	20	20	25	28	25	15	06	24	20	29	24	23

**Figure 4.** Summary diagram of sensibility of isolated strains.

is indeed in this age group that we eat everything without much control, and believe in the strength of teeth like iron. Regarding sex, it is the female sex that was more represented with a sex ratio equal to 1.5. However, this result differs from those obtained by [9]. Indeed, out of a total of 200 decayed teeth of all sexes, 128 were male versus 72 females with a sex ratio of 1.7. Which consequently suggests that dental caries is not linked to sex.

Furthermore, the strains were identified for the most part with a confidence level of 99.9%, thus indicating the purity of the strains studied, except for six strains whose confidence level fluctuates between 50 and 85. Also, this identification made it possible to divide into 5 families with greater repetitiveness in Enter-

obacteriaceae followed by *Pseudomonas* and then *Staphylococcus*. The majority presence of Enterobacteriaceae in carious teeth would be due to the hygiene conditions not respected because these bacteria are generally of intestinal origin. *Pseudomonas* and *Staphylococcus* are found there due to an environment conducive to their growth. Analysis of the antibiotic resistance profile showed that all strains studied showed resistance to some antibiotics and multi-resistance to others. Regarding the *Pseudomonas* and Enterobacteriaceae families, all strains showed resistance to piperacillin tazobactam (TZP) and amoxicillin (AMC).

For the *Staphylococcus* family, all strains were resistant to tetracycline (TET). However, only the *Staphylococcus* and Enterobacteriaceae families showed multi-resistance to the antibiotics tested, including piperacillin tazobactam (TZP), amoxicillin (AMC), tetracycline (TET), tobramycin (TOB) and erythromycin (ERY). This observation suggests that piperacillin tazobactam (TZP), amoxicillin (AMC), tetracycline (TET) would be the molecules most prescribed by dentists and clinicians in antibiotic therapy. In addition, this multi-resistance could be attributed to the fact that the large family of beta-lactams, which include penicillins and cephalosporins, is the most used in first-line anti-infective therapy because it is the most diverse and the least toxic [10]. These results would agree with those of [11] who showed a prescription of nearly 98.20% of beta-lactams as first-line during their work on antibiotics. The same is true of the work of [12] and [13] respectively in the United States and Brazil who made the same observations. It seems obvious that this strong prescription of antibiotics is often without necessity the fundamental cause of this resistance and bacterial multi-resistance of these strains studied. It should be remembered that the prescription of antibiotics to be prophylactic should not be systematic in patients suffering from general pathology. This is inappropriate. Indeed, only in patients at high risk of infective endocarditis or any dental procedure involving manipulation of the gum, the periapical region of the tooth and in the event of extraction [14]. In view of this, the fight against bacterial resistance or multi-resistance would not be a magic fact. It is and remains above all a paradigm shifts both at the level of patients and at the level of practitioners in our various health centers. We need intense awareness now to raise awareness about the use of antibiotics. Thus, human shipwrecks could be avoided for antibiotic therapy for future generations.

5. Conclusion

At the end of this study, it should be noted that dental pathology is not linked to age or gender. It is a disease that can be contracted at any age. The bacterial germs involved in this study are mainly Enterobacteria, *Pseudomonas* and *Staphylococcus*. The antibiogram carried out revealed resistance on the one hand and multi-resistance on the other hand on the different strains identified. This observation is therefore a challenge not only for practitioners in dental practice but also for patients who must be careful about the use of antibiotics. It should be remembered here that the fight against the phenomenon of bacterial resistance or even multi-

resistance is a real fact and concerns everyone at whatever level. In view of these convincing results, this study could be extended to other hospital centers such as school medical centers in the region, in order to increase the size of the sample to be analyzed and provide statistics on a regional scale.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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