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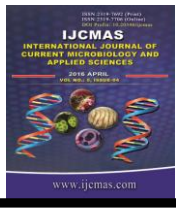

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Ecology and Bacterial Resistance Level in the Tooth Decay: Case of Patients Consulted in a General Hospital in Abidjan (Côte D'ivoire)

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A B S T R A C T

The apparition of bacteria resistant to the antibiotics and their implication in human infections constitute a real problem of public health. The objective of this survey was to determine the resistance level of the bacteria to the antibiotics used in the treatment of the tooth decay. The strain isolation has been performed respectively on BEA (Bile Esculine Agar), Chapman, Cetrimide, EMB (Eosine and to the Bruise of Methylene), MRS (Man Rogosa Sharpe) to the cool blood, MH (Müller Hinton) to cooked blood added polivitex and Colombia to the fresh blood added nalidixic acid and colistine. For their identification, the macroscopic and, microscopic analysis and different tests have been achieved. *Enterococcus sp*, *Staphylococcus aureus*, *Staphylococcus* negative coagulase, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Lactobacillus* sp, *Streptococcus mutans* and *Streptococcus* sp bacterial strains have been isolated with different phenotypes. Of all studied species, *Lactobacillus* sp and *Streptococcus mutans* have been confirmed responsible for the tooth decay with 22.2%. This survey showed that the oral dental cavity contains a diversity of bacteria of which most are resistant to the families of used antibiotics.

Introduction

Antibiotics apparition to the twentieth century was a real revolution in medicine and dragged a reduction of mortality associated to bacterial origin infectious diseases bacterial origin. Their abusive use

entailed the resistance of these bacteria to the antibiotics and therefore, the increase of the therapeutic failures (Willemsen and al., 2009; Rabaud and Birge, 2015). The emergence of the bacteria resistant to the antibiotics can entail the increase of the morbidity, and in some cases, mortality. Besides, one of the consequences bound to this bacterial resistance is the increase of the treatment costs (Karl, 2002). Otherwise the tooth decay that is a pathology due to bacteria notably *Lactobacillus* and the *Streptococcus*, is classified to the 3rd rank of the world curses (Gondian; 2003). Indeed, according to the WHO (2012), this infectious deases affects between 60% to 90% of the pupils and close to 100% of the adults. However very few data exist on the ecology and the rate of bacteria resistance responsible of the tooth decay in Côte d'Ivoire. The objective of this survey is to put in evidence the ecology and the resistance level of bacteria used in the treatment of the tooth decay.

Materials and Methods

Patients

The strains were isolated from patients of all ages received first consultation at the General Hospital of Abobo Nord, after informed and written consent. For children whose age was lower than 15 years, the consents have been required for accompanying parents.

Methods

Bacterial Sampling

Twenty (20) samples in the decayed teeth have been achieved on the patients in the period from August to October 2014. A Dacron type dry swab has been introduced in the hollow of the tooth decayed to take the produced secretions. The swabs have been introduced then in the physiological water contained in screw and sealed tubes. The samples have been kept in one jar until the laboratory.

Isolation of Bacterial Strains

For the isolation of strains, swabs were seeded on different selective media. The isolation of *Streptococcus* has been performed on Columbia agar blood added to fresh nalidixic acid and colistin (15 mg) respectively, then on the MH agar supplemented with cooked polivitex blood. *Lactobacllus* were isolated on MRS agar with fresh blood, *Staphylococcus* on mannitol salt agar, *Enterobacteriaceae* on EMB, *enterococci* on BEA agar and *Pseudomonas* on cetrimide agar. All petri dishes have been then incubated at 37 ° C for 24 hours. The Petri dishes Search *Streptococcus* and *Lactobacillus* have been incubated in a steam room under 5% CO_2.

Identification (Api and ID 32)

After macroscopic and microscopic analysis, the different strains have been identified according on the following tests:

The Pastorex Strep over test ((Bio-Rad) has been performed for Gram positive cocci bacteria chainlets, isolated on agar MH cooked blood supplemented with Polivitex Colombia and blood supplemented with nalidixic acid and colistin. For confirmation of *Streptococcus mutans* presence, the mannitol test has been performed (Freney *et al.*, 2000).

The API 20 E (Biomerieux) followed by the rack of Leminor (Freney *et al.*, 2000) have been used for the identification of bacterial strains Gram negative bacilli, negative oxidase obtained on EMB.

Strains Gram-positive cocci in clusters, obtained on Chapman agar have been identified by Pastorex Staph plus test (Bio-Rad). *Staphylococcus aureus* confirmation has been made by the test in hydrochloric acid (Freney *et al.,* 2000).

Gram positive Bacteria cocci in short silver chain, producer esculin on BEA agar with negative catalase is Characteristic of *Enterococcus* (Isenberg *et al.,* 1970).

Bacterial strains with a green pigment on cetrimide agar, Gram negative bacilli, oxidase positive, have been identified by the gallery API 20 NE (Biomerieux). *Pseudomonas aeruginosa* presence has been confirmed by the temperature test (37 ° C and 44° C) (Freney *et al.,* 2000).

Bacteria bacilli gram positive, catalase and oxidase negative, isolated on MRS agar fresh blood, have been identified by API 50 CH gallery

Isolated Strains Antibiogram Realization

The antibiogram has been carried out by diffusion of discs and has been interpreted according to the criteria described in Ca-SFM 2013. The list of antibiotics used with their charge is as follows: Teicoplanin (TEC; 30 µg), Vacomycin (VA; 30 µg), Lincomycin (L; 15 µg), Nitrofurane (FT; 300 µg), strog Kanamycin (KAN; 1000 µg), strong Gentamycin (GEN; 500 µg), Ampicillin (AM; 10 µg), Cefalotin (CF; 30 µg), Cefoxitin (FOX; 30 µg), Cefepime (FEP; 30 µg), Ceftriaxone (CRO; 30 µg) and Spiramycin (SP; 100 µg), Fusidic acid (FA; 10 µg), Fosfomycin (FOS; 30 µg), Gentamycin (GM; 15 µg), Pristinamycin (PT; 15 µg), Pefloxacyn (PEF; 5 µg), ciprofloxacin (CIP; 5 µg), **S**ulfamide (SSS; 200 µg), Minocycline (MNO; 30 µg), Tetracycline (TE; 30 µg), Erythromycin (E; 15 µg), Imipenem (IPM; 10 µg), Meropenem (MEM; 10 µg), Aztreonam (ATM; 30 µg), Cefotaxime (CTX; 30 µg).

Results and Discussion

Twenty five (25) bacterial strains have been isolated whose distribution is the following: (figure 1).

The strains sensitivity opposite the antibiotics according to the species is consigned in tables III-VIII.

The different phenotypes of resistance of the strains after the realization of the antibiogram were consigned in the table IX.

The germs as, *Enterococcus* sp, *Staphylococcus aureus*, *Staphylococcus negative coagulase*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Lactobacillus* sp, *Streptococcus mutans* and *Streptococcus* sp were the main bacteria isolated in the dental cavity of the patients. These germs are part of the flora of the oral dental cavity since the birth (Könönen, 2000) and vary according to age (Könönen and al., 1994), the most predominant at the time of a tooth decay are the Streptococci (Aas and al., 2008). The predominance of the Streptococcus and lactobacillus has been demonstrated at the time of the works of Barsamian-Wunsch and al. in 2004, in which a comparative survey between the different methods of isolations of the *Streptococcus mutans* and the Lactobacilluses has been achieved. These results are in concordance with the our, in which a number raised from Streptococcus (40%) and of *Lactobacillus* sp (16%) has been gotten. This predominance of Streptococcus could explain itself by the insufficient hygiene, of the non treated caries, the accumulation of the dental plate.

Table.III Strains Sensitivity of *Enterococcus Sp* Vis-a-Vis Antibiotics

	Strains code of *Enteroc.* sp		
Antibiotics	999C/14	1013C/14	1160C/14
Teicoplanin	S	S	-
Lincomycin	I	-	S
Nitrofuranes	R	S	S
Kanamycin	S	-	S
Vacomycin	S	S	S
Strong Gentamycin	-	I	-
Ampicillin	S	S	R
Spiramycin	S	S	S
Cefalotin	S	R	R
Cefoxitin	R	R	R
Cefepime	S	R	R
Ceftriaxone	S	R	R

S:Sensitive;I:Intermediate;R:Resistant

Table.IV Strains Sensitivity of *Staphylococcus* Vis-a-Vis Antibiotics

	Strains code of		
	S. aureus		*S.* coag. nég
Antibiotics	1000C/14	1159C/14	1014C/14
Cefoxitin	R	S	S
Cefalotin	R	-	S
Ceftriaxone	R	S	S
Cefepime	R	S	S
Ampicillin	R	S	S
Vacomycin	S	S	S
Fosfomycin	S	S	-
Teicoplanin	S	-	S
Gentamicin	R	S	S
Pristinamycin	S	-	S
Fusidic acid	S	-	-
Erythromycin	-	R	S
Ciprofloxacin	-	-	S
Kanamycin	-	S	-
Minocyclin	-	R	-
Tetracyclin	-	R	-
Spiramycin	I	S	S

S:Sensitive;I:Intermediate;R:Resistant

Table.V Sensitivity Strains of *Streptococcus* Vis-a-Vis Antibiotics

	Strains code of									
	Streptococcus mutans			*Streptococcus* sp						
Antibiotics	1135C /14	1142 C /14	1143 C/14	1136 C/14	1137 C/14	1138 C/14	1139 C/14	1140 C/14	1141C /14	1144 C/14
Pristinamycin	S	S	S	S	S	S	S	S	S	S
Lincomycin	R	R	R	S	S	S	R	S	S	S
Erythromycin	R	R	R	S	S	R	R	R	R	R
Tetracyclin	R	R	R	R	R	R	R	R	R	R
Ampicillin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Spiramycin	R	R	R	S	S	S	R	S	S	S
Cefalotin	S	S	S	S	S	S	S	S	I	S
Cefoxitin	S	S	S	S	S	S	S	S	R	S
Cefepime	S	S	S	S	S	S	S	S	S	S
Ceftriaxone	S	S	S	S	S	S	S	S	S	S

S:Sensitive; I:Intermediate; R:Resistant; NS : not smell

Table.VI Strains Sensitivity of the of *Pseudomonas aeruginosa* Vis-a-Vis Antibiotics

	Stains code of *P. aeruginosa*
Antibiotics	1001C/14
Ciprofloxacin	S
Gentamicin	S
Imipenem	S
Cefepime	S
Cefalotin	R
Cefoxitin	R
Ceftriaxone	R
Ampicillin	R
Spiramycin	R

S:Sensitive; I:Intermediate; R:Resistant

Table.VII Strains Sensitivity of *Klebsiella pneumoniae* Vis-A-Vis Antibiotics

	Stains code of *K. pneumoniae*			
Antibiotics	1015C/14	1016C/14	1017C/14	1145C/14
Cefoxitin	S	S	S	S
Cefotaxime	S	S	S	S
Cefepime	S	S	S	S
Amox. + clavul acid.	S	S	S	S
Imipenem	S	S	S	-
Aztreonam	S	S	S	S
Cefalotin	S	S	S	S
Ceftriaxone	S	S	S	S
Meropenem	-	-	-	S
Ampicillin	R	R	R	R
Spiramycin	R	R	R	R

S:Sensitive; R:Resistant

Table.VIII Strains Sensitivity of *Lactobacillus acidophilus* Vis-A-Vis Antibiotics

	Stains code of *Lactobacillus* sp			
Antibiotics	1131C/14	1132C/14	1133C/14	1134C/14
Ampicillin	S	S	R	S
Spiramycin	S	S	S	S
Cefalotin	S	S	S	S
Cefoxitin	I	S	I	S
Cefepime	S	S	S	S
Ceftriaxone	S	S	S	S

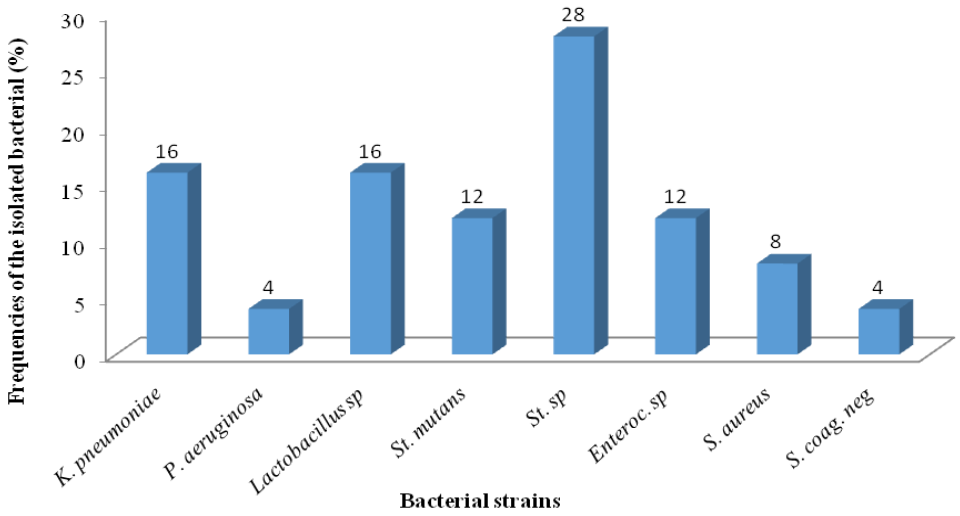
S:Sensitive; I:Intermediate; R:Resistant

Table.IX Phenotypes of Resistance to Antibiotics of the Stocks after Antibiogramme

Code	Bacterial strain	Biological product	Phenotype
999C/14	*Enterococcus* sp	Dental decay	Resistance of low level to the kanamycine
1000C/14	*Staphylococcus aureus*	Dental decay	KTG, Meti-R
1001C/14	*Pseudomonas aeruginosa*	Dental decay	Savage
1013C/14	*Enterococcus* sp	Dental decay	Resistance of low level to gentamicin
1014C/14	*Staphylococcus* caog. negative	Dental decay	Savage
1015C/14	*klebsiella pneumoniae*	Dental decay	Savage
1016C/14	*klebsiella pneumoniae*	Dental decay	Savage
1017/14	*klebsiella pneumoniae*	Dental decay	Savage
1131C/14	*Lactobacillus* sp	Dental decay	Savage
1132C/14	*Lactobacillus* sp	Dental decay	Savage
1133C/14	*Lactobacillus* sp	Dental decay	Savage
1134C/14	*Lactobacillus* sp	Dental decay	Savage
1135C/14	*Streptococcus mutans*	Dental decay	MLSb (SDBLAC)
1136C/14	*Streptococcus* sp	Dental decay	Savage (SDBLAC)
1137C/14	*Streptococcus* sp	Dental decay	Savage (SDBLAC)
1138C/14	*Streptococcus* sp	Dental decay	SDBLAC
1139C/14	*Streptococcus* sp	Dental decay	MLSb inductible (SDBLAC)
1140C/14	*Streptococcus* sp	Dental decay	SDBLAC
1141C/14	*Streptococcus* sp	Dental decay	SDBLAC
1142C/14	*Streptococcus mutans*	Dental decay	MLSb (SDBLAC)
1143C/14	*Streptococus mutans*	Dental decay	MLSb (SDBLAC)
1144C/14	*Streptococcus* sp	Dental decay	SDBLAC
1145C/14	*Klebsiella pneumoniae*	Dental decay	Savage
1159C/14	*Staphylococcus aureus*	Dental decay	Savage
1160C/14	*Enterococcus sp*	Dental decay	Savage

KTG: Resistance to Kanamycine, Tobramycin and Gentamycine; Meti-R: Resistance to Meticilline;
MLS: Resistance to Macrolides, Lincosamines, and Streptogramines; b: type of resistance (inductible b);SDBLAC: Susceptibility decreased to beta-lactamines; AM: Ampicilline; SP: Spiramycine;
CF:Cefalotine;FOX:Cefoxitine;FEP:Cefepime;CRO:Ceftriaxone;AMC:Amoxicilline + acid clavulanic;(-):Not tested; N:A number of stocks tested; NS: Not smell

Figure.1 Isolated Bacterial Strains Frequencies



The infection will be able to make itself through the consumption of food contaminated by these germs or by saliva (Grindefjord and al., 1995). The action conjugated of *Streptococcus* and *Lactobacillus* in the lactic acid production to the level of the tooth decay, would slow down the proliferation of the other isolated germs (You and al., 1999).

The realization of the antibiogram of the isolated strains allowed to observe phenotypes of different resistances. The antibiotics use in the oral care, more precisely in the tooth decay, that is to say β-lactamines, constituted of the different penicillins and macrolides (Sixou and Monsarrat, 2010), present different natural resistances to the antibiotics screws has screw of the bacteria recovered in this affection (Meyohas and Pacanowski, 2007,; Sylvie Carle, 2009,; Habib and al., 2009). Notice also that all strains of *Streptococcus mutans*, precursor of the tooth decay, were resistant to the spiramycin, antibiotic used in the treatment of this affection. This resistance of the germs, could explain themselves either by a genetic resistance, crossing either chromosomal hard has the prescription raised of these molecules in Africa (Dosso and al., 2000), and by their abusive use.

The present survey showed that the oral cavity is rich in bacteria. These are the *Enterococcus* sp, *Staphylococcus aureus*, negative-coagulase *Staphylococcus*, *Pseudomonas aeruginosa*, *Klebsiellas pneumoniae*, *Lactobacillus* sp, *Streptococcus mutans* and *Streptococcus* sp with different phenotypes during an oral infection. These bacterial strains would limit the effective treatment of tooth decay because of their resistance to the prescribed antibiotics. It is why, we recommend their use with moderation.

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