Antibiogram types of bacterial isolates from dental caries patients attending clinic at a teaching hospital in Nigeria

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Abstract

Around the world dental caries is amongst the commonest disease affecting all age groups and yet seen by all as less common and severe; especially among Africans. Worrisome, our recent study showed isolated dental caries bacteria to be resistant to available antibiotics. In this study, antibiogram typing of bacteria isolated from dental caries of patients attending the dental clinic at Irrua Specialist Teaching Hospital, Nigeria were investigated. The study involves 223 bacteria positive samples (Streptococcus mutans = 150; Streptococcus sobrinus = 36; Lactobacillus acidophilus = 22; Streptococcus salivarius = 10; Streptococcus mitis = 4) collected and screened against 24 antibiotics divided into 8 groups of 3 each. Antibiogram study was conducted using Ajumali’s mnemonic coding and the results were presented in tables. The Ajumali’s mnemonic coding showed that no two strains of Streptococcus salivarius and Streptococcus mitis were repeated. However, 18.8%, 43.71%, and 61.11% of Lactobacillus acidophilus, Streptococcus mutans, and Streptococcus sobrinus strain respectively presented repeated Ajumali’s mnemonic codes. The “00000005, 02000005, 03010005, 01010005 and 63112005” mnemonic codes appear in both S. mutans and S. sobrinus isolates, “02000004” appears in both L. acidophilus and S. mutans, 03000005 appears in both S. salivarius and S. mutans and 03110005 in S. mitis and S. sobrinus. The repetition of mnemonic codes from L. acidophilus, S. mutans, and S. sobrinus isolates and the appearance of codes between isolates indicate that some isolates may have strains with phenotypical similarities.

Keywords: Antibiogram types; Bacterial isolates; Dental caries; Ajumali’s mnemonic coding

1. Introduction

The oral cavity is usually within a certain temperature, moisture contains different nutritional compounds including carbohydrates, lipids, and proteins and shelters the growth of normal flora and some pathogenic bacteria [1, 2]. The growth and multiplication of bacteria colonizing the surface of teeth are the primary etiological agent of dental caries [3] and is recognized as one of the most infectious diseases worldwide [4, 5].

Actively growing bacterial colonies surrounding teeth area initiate the formation of biofilm by agglomerating into a long chain to form complex heterogeneous structures with the aid of their enzymes and excretory products [2]. The formed biofilm can resist different antibiotics, alcohol, and different bacteriocidal and bacteriostatic agents at a concentration of 10 to 100 times than that needed to destroy and is also exceptionally escape phagocytosis that helps to exist inside the host’s oral cavity and makes it extremely difficult to kill [6]. These are the mechanisms that make the biofilm difficult to destroy when the therapeutic drugs are used in dental diseases [3].

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Dental caries usually demolishes the enamel and dentin by bacterial activity [7-10]. Globally, dental caries are a common chronic condition among people of all ages [11] and evidence has suggested linkage with overall health and utilization of dental care can cut down health care cost 12, 13. It is now avowed that the formation of bacterial biofilm is responsible for a variety of human diseases such as osteomyelitis, middle ear infections, dental caries, medical instrument and device-related infections, native valve endocarditis, ocular implant infections, and chronic lung infections [14]. Despite this evidence of therapeutic resistivity, the commonest of dental caries as well as its links with other diseases, oral health remains optional for most populations. In addition, the antibiotic typing for bacteria isolate; especially for dental caries bacterial, has not been extensively studied. Yet it has long been documented that typing methods, like the bacteriophage typing of Staphylococcus aureus and Listeria monocytogenes [15, 16], serotyping of Salmonella spp. and Escherichia coli [17, 18], or biochemical typing of Enterobacteriaceae [19], have historically been important and contributed to the understanding of natural history and epidemiology of infections caused by strains of bacterial species [20]. It is therefore the aim of this study to analyze the antibiogram typing of bacterial isolates from dental caries isolates of patients attending a dental clinic in a teaching hospital in Nigeria using Ajumal’s mnemonic coding method.

2. Material and methods

2.1. Study location and population

The study was carried out in Irrua Specialist Teaching Hospital (I.S.T.H), Irrua in Esan Central Local Government Area of Edo State, Nigeria which lies at Latitude 6.45°N and Longitude 6.15°E. This hospital is a tertiary health facility. The subjects included all patients attending the dental clinic in the Hospital.

2.2. Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of the Ambrose Alli University, Ekpoma (assigned number: 10/17 (001/17). The study was conducted according to the WMA declaration of Helsinki-Ethical principles for medical research involving human subjects.

2.3. Sample Collection and Processing

Three hundred and forty samples of suspected cases of dental caries were collected during the period of study. Following the recommendation of Dragica et al. [21], sterile dental forceps and sterile swab sticks, were used aseptically to collect samples, by scraping or swabbing the suspected caries lesion. All samples were cultured within 1 hour of collection in the Medical Microbiology Laboratory of Irrua Specialist Teaching Hospital. However, only 222 samples were positive for bacteria colonization and were analyzed.

2.4. Morphological and biochemical identification of isolates

The culture characteristics of isolated bacteria such as size, shape, hemolysis, pigmentation, and consistency were noted according to Cheesbrough [22]. The biochemical properties of the isolates were studied according to Bergey’s Manual of Determinative Bacteriology.

2.5. Antibiotic Susceptibility Test

The susceptibility of the bacterial isolates to antibiotics were determined by single disc agar diffusion method as previously described by Cruikshank et al. [23] and Ochei and Kolhatkar [24] and 0.5 Mcfarland standard was used to standardize the inoculum to the density of bacterial suspension of 1.5 x 10^8 (CFU/ml). Streptococci isolates were seeded on chocolate agar, while Lactobacilli species were seeded on Mueller Hinton agar for antimicrobial sensitivity testing. Furthermore, before the commencement of the experiment, control for the antibiotics was done against Staphylococcus aureus ATCC 25923 at 37°C for 24-48hours. The antimicrobial sensitivity plates were also controlled before usage by sterility test as described by Ochei and Kolhatkar [24].

The selection of antibiotics for this study was done by dividing 24 antibiotics into eight different groups of 3 antibiotics each per group, according to one or more of these criteria; mode of action, similarities, usage in the community, and generation of discovery as in Orhue et al [27]. In each group, 3 antibiotics were chosen, and were 2 antibiotics were available, another antibiotic that was odd to the group was used to complete the triplet arrangement. Each triplet antibiotics in each group were arranged in ascending order of their molecular weight viz;
2.5.1. **Group 1**

Ampicillin, Amoxicillin, and Imipenem. These are bacteria cell wall synthesis inhibitors. Imipenem has the advantage of being stable in the presence of β-lactamase (both penicillinase and cephalosporins) produced by many multiple drug-resistant Gram-negative bacteria. Ampicillin is a broad-spectrum aminopenicillin, while amoxicillin is a moderate spectrum, bacteriolytic, β-lactam antibiotic in the aminopenicillin family, and also it is better absorbed, following oral administration than other β-lactam antibiotics.

2.5.2. **Group 2**

Augmentin, Unasyn, and Flucloxacillin: Augmentin (amoxicillin/clavulanic acid) is a combination consisting of amoxicillin, a β-lactam antibiotic, and potassium clavulanate, a β-lactamase inhibitor. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β-lactamase. Unasyn (ampicillin/sulbactam) is a combination of the common penicillin-derived antibiotic ampicillin and sulbactam, an inhibitor of bacterial beta-lactamase. The addition of sulbactam to ampicillin enhances the effects of ampicillin and is active against a wide range of bacterial. Flucloxacillin is a broad-spectrum with a fluorinated side chain, and it has activity against beta-lactamase-producing organisms such as *Staphylococcus aureus*.

2.5.3. **Group 3**

Chloramphenicol, Tetracycline, and Co-trimoxazole: Cotrimoxazole (Septrin) is a folic acid inhibitor while the other two are protein synthesis inhibitors. Here, they represent the most commonly used (abused) antimicrobials in the community.

2.5.4. **Group 4**

Erythromycin, Clarithromycin, and Azithromycin. These 3 antibiotics belong to the class of antibiotics called the Macrolides. They are inhibitors of protein synthesis and are bacteriostatic with a broad spectrum of activity against many Gram-positive bacteria such as *Streptococcus pneumoniae* and few Gram-negative bacteria. Azithromycin and newer macrolides such as clarithromycin can be regarded as advanced generation macrolides compared with erythromycin.

2.5.5. **Group 5**

Gentamicin, Streptomycin, and Neomycin: These three aminoglycosides are broad-spectrum protein synthesis inhibitors, and they exhibit concentration-dependent bactericidal activity against most Gram-negative aerobic and facultative anaerobic bacilli, but not against Gram-negative anaerobes and most Gram-positive bacteria.

2.5.6. **Group 6**

Cephalexine, Cefuroxime, and Cefotaxime: These are cephalosporins class of antibiotics and together with cephemycins, they constitute a subgroup of β-lactam antibiotic called cephems. Cephalexin belongs to the 1st generation cephalosporins and is active predominantly against Gram-positive bacteria, and other generations have increased activity against Gram-negative bacteria, but often with reduced activity against Gram-positive bacteria. Cefuroxime and cefotaxime belong to the 2nd and 3rd generation cephalosporin respectively.

2.5.7. **Group 7**

Ciprofloxacin, Pefloxacin, and Ofloxacin. These three antibiotics belong to the Quinolones or Fluoroquinolone class of antibiotics. These three Quinolones act by inhibiting the DNA gyrase, and they have broad-spectrum activity.

2.5.8. **Group 8**

Metronidazole, Lincomycin, and Clindamycin. Metronidazole is anti-protozoan but is also active against anaerobic and facultative anaerobic bacteria. It inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. Lincomycin and Clindamycin belong to the class of antibiotic known as the Lincosamides. Lincosamides prevent bacteria from replicating by interfering with the synthesis of proteins. Clindamycin is very active against anaerobes.

2.6. **Antibiogram typing using Ajumali’s Method of Pneumonic Coding**

As can be seen on the result table, the sensitive result was scored as (+) while resistance was recorded as (-). The three antibiotics in each group were given numerical values of 1, 2, and 4. A perfect sensitivity to the three antibiotics will give a summation of $1+2+4 = 7$. Complete resistance to the three antibiotics will give a summation of $0+0+0 = 0$. Other
values are obtained by adding up these arbitrary numerical values; in which case an isolate can receive a score of 0 - 7 in each triplet segment, together which then gives an eight-digit numerical value as the antibiogram type. NB: this is an adaptation of Ajumali’s method of pneumonic coding [25].

3. Results and discussion

The ability to quickly and reliably differentiate among related bacterial isolates is essential for epidemiological surveillance and is an endeavor as old as the discipline of bacteriology itself [20]. With the advance of molecular technology, typing methods with high versatility, type ability, reproducibility, and discrimination have been developed but they are never available in many hospitals because of the complex technology and costs for their implementation [26]. However, patterns of susceptibility to antimicrobial agents have been used widely for typing purposes, due to their ready availability, simplicity, and relatively cheap to carry out [26]. In the present study, 222 isolates of bacteria positive caries patients consisting of Lactobacillus acidophilus (n = 22), Streptococcus salivarius (n = 10), Streptococcus mitis (n = 4), Streptococcus sobrinus (n = 36) and Streptococcus mutans (n = 150) were examined. These isolates were screened against 24 antibiotics divided into 8 groups of 3 antibiotics and the antibiogram typing was determined using Ajumali’s mnemonic coding as presented in tables 1 to 5. The Ajumali’s mnemonic coding showed that no two strains of Streptococcus salivarius (table 2) and Streptococcus mitis (table 3) presented repeated code. This absence of duplicate code from S. salivarius and S. mitis appears to follow what Orhue et al. [27] reported for Staphylococcus spp (Staphylococcus aureus, Streptococcus faecalis, and Staphylococcus saprophyticus) in urine samples collected from suspected cases of urinary tract infections in the University of Benin Teaching Hospital, Nigeria. According to Orhue et al. [27], the absence of repeated code in S. salivarius and S. mitis makes each of the isolates phenotypically different from one another, even though they are of the same species and by implication, indicating a very higher resolving strain differentiation.

However, several strains of Lactobacillus acidophilus (table 1), Streptococcus sobrinus (table 4), and Streptococcus mutans (table 5a, b, and c) presented repeated codes. For Lactobacillus acidophilus (table 1) there were duplicates of repeated codes (01000004 and 02000004) resulting in 18.18% repeated codes. Streptococcus sobrinus (table 4) presented repeated codes of 61.11% with 03110005 and 01010005 repeated 4 times, 03010005 and 4312005 repeated 3 times and 63112005, 01000005, 02000005, and 03112005 duplicates. Streptococcus mutans (table 5a, b, and c) presented 43.71% repeated codes with 00000005 repeated 9 times, 03010005 and 23010005 repeated 5 times, 03000005, 02000005 and 63112005 repeated 4 times, 01010005, 02010004, 02010005, 22010005, 63110005 repeated 3 times and 10 duplicated codes. According to Orhue et al. [27], the presence of repeated codes in L. acidophilus, S. sobrinus, and S. mutans indicate that some of the isolates are phenotypically similar.

Figure 1 shows a set presentation of mnemonic codes appearing between isolated bacteria. Interestingly, the “02000004 mnemonic code” appears in both L. acidophilus and S. mutans while “00000005, 02000005, 03010005, 01010005 and 63112005” mnemonic codes appear in both S. mutans and S. sobrinus isolates, “02000004” appears in both L. acidophilus and S. mutans, 03000005 appears in both S. salivarius and S. mutans and03110005 in S. mitis and S. sobrinus. Antibiogram typing has been used in clinical microbiology as a first-line method for identifying cases of bacterial cross-transmission [20] and to date still useful even if it has several practical limitations that makes it not suitable for comprehensive studies of bacterial population structure and dynamics, but very critical, endeavors of infection control and surveillance [28, 29].
Table 1 Sensitivity and antibiogram types of L. acidophilus isolated from dental caries cases in ISTH Irrua

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<th>Amoxicillin</th>
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<th>Erythromycin</th>
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Table 2 Sensitivity and antibiogram types of S. salivarius isolated from dental caries cases in ISTH Irrua

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Keys: - indicates resistance, + indicates sensitive.
### Table 3 Sensitivity and antibiogram types of *S. mitis* isolated from dental caries cases in ISTH Irrua

| Bacterial isolates | Ampicillin | Amoxicillin | Imipenem | Augmentin | Unasyn | Fluoroxacillin | Chloramphenicol | Tetracycline | Cotrimoxazole | Erythromycin | Clarithromycin | Azithromycin | Gentamycin | Streptomycin | Neomycin | Cephalaxin | Cefuroxime | Cefotaxime | Ciprofloxacin | Pefloxacin | Ofloxacin | Metronidazole | Lincomycin | Clindamycin |
|-------------------|------------|-------------|----------|-----------|--------|----------------|-----------------|--------------|---------------|-------------|---------------|-------------|------------|-------------|---------|-----------|-------------|-------------|-------------|-------------|--------------|------------|-------------|
| 1                 | -          | -           | +        | +         | +      | -              | -               | -            | -             | -           | -             | -           | -          | -           | -       | -         | -           | -           | -           | -           | -            | -          | -           | -            |
| 2                 | -          | +           | +        | +         | +      | -              | -               | -            | +             | -           | -             | -           | +          | -           | -       | +         | +           | +           | +           | +           | +            | +          | +           | +            |
| 3                 | -          | -           | -        | +         | +      | -              | +               | -            | -             | -           | -             | -           | -          | -           | -       | +         | +           | -           | +           | +           | -            | +          | -           | +            |
| 4                 | -          | -           | +        | +         | +      | -              | -               | -            | -             | -           | -             | -           | -          | -           | -       | +         | +           | -           | +           | +           | -            | +          | -           | +            |

**Keys:** - indicates resistance, + indicates sensitive.

### Table 4 Sensitivity and antibiogram types of *S. sobrinus* isolated from dental caries cases in ISTH Irrua

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<th>Imipenem</th>
<th>Augmentin</th>
<th>Unasyn</th>
<th>Fluoroxacillin</th>
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<th>Tetracycline</th>
<th>Cotrimoxazole</th>
<th>Erythromycin</th>
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<th>Cefotaxime</th>
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Table 5a Sensitivity and antibiogram types of *S. mutans* isolated from dental caries cases in ISTH Irrua

| Bacterial isolates | Ampicillin | Amoxicillin | Imipenem | Augmentin | Unasyn | Phloxacin | Chloramphenicol | Tetracycline | Cotrimoxazole | Erythromycin | Azithromycin | Gentamycin | Streptomycin | Neomycin | Cephalaxin | Cefuroxime | Cefotaxime | Ciprofloxacin | Pefloxacin | Ofloxacin | Metronidazole | Lincomycin | Clindamycin | TYPES |
|-------------------|------------|-------------|-----------|-----------|--------|-----------|----------------|--------------|---------------|--------------|--------------|------------|--------------|---------|-----------|------------|------------|----------------|-------------|-----------|----------------|------------|------------|-------------|-------------|
|                   | 1  2  4  1  2  4  1  2  4  1  2  4  1  2  4  1  2  4  1  2  4 | - | + | + | + | - | + | - | + | - | - | - | - | - | - | - | - | - | + | + | - | 61110005 | 00000005 | 77110005 | 00010005 | 02010005 | 02012405 | 03312627 | 02010005 | 02130005 | 02010005 | 02000005 | 06711007 | 02000004 | 02042005 |
| 22 | - | + | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | 22032001 |
| 23 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 00000005 |
| 24 | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - | - | 03110606 |
| 25 | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | 20010005 |
| 26 | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 43100005 |
| 27 | - | + | + | + | + | - | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | + | - | - | 67270407 |
| 28 | - | + | + | + | + | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 63030407 |
| 29 | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 07100005 |
| 30 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 23010005 |
| 31 | - | + | - | + | + | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 27112405 |
| 32 | + | + | + | + | + | - | - | - | + | - | - | - | + | - | - | - | - | + | - | - | - | + | - | - | 73012407 |
| 33 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 03010005 |
| 34 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 22000001 |
| 35 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 22010005 |
| 36 | - | + | + | + | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 67010405 |
| 37 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 02000004 |
| 38 | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 63112005 |
| 39 | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 63040407 |
| 40 | - | + | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 27010001 |
| 41 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 00000007 |
| 42 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 23000005 |
| 43 | - | + | + | + | + | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 67072407 |
| 44 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 00000005 |
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| 46 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 00000005 |
| 47 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 02010004 |
| 48 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 23000004 |
| 49 | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 21110607 |
| 50 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 02000005 |

Keys: - indicates resistance, + indicates sensitive.
Table 5b  Sensitivity and antibiogram types of *S. mutans* isolated from dental caries cases in ISTH Irrua continue

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Keys: - indicates resistance, + indicates sensitive.
In the present study, it was observed that S. mutans isolates share some coding similarities with *S. salivarius, S. sobrinus,* and *L. acidophilus* while *S. sobrinus* and *S. mitis* also show a code similarity. In fact, *S. mutans* and *S. sobrinus* share about 5 phenotyping codes. The repetition of mnemonic codes within *L. acidophilus, S. mutans,* and *S. sobrinus* isolates and the appearance of codes between isolates indicate that some isolates may have strains with phenotypical similarities. Although in some cases it is a very useful prerequisite, it is generally accepted that phenotyping cannot usually stand-alone because the rate of genetic exchange within many bacterial species that a given phenotype may not always reflect evolutionary history [20]. Based on this assertion, two isolates that are phenotypically identical according to antibiogram typing might be discrete and vice versa. For these reasons, over the past two decades phenotyping has been largely replaced by genotypic or ‘molecular’ typing [30-35].

4. Conclusion

Typing methods are used to study the spread and population dynamics of bacteria and other microorganisms in clinical and environmental settings, the antibiogram typing results of the present study showed that some dental caries bacteria might be phenotypically similar. While antibiogram typing using antibiotics susceptibility pattern may be simple, rapid, and readily available, and especially suitable for hospital laboratories with no sophisticated typing facilities, antibiogram typing with the present method can be used for bacteria surveillance, epidemiological analysis, and infection control purposes. We however recommend that other methods be carried out to investigate the reported phenotypic similarity between dental caries bacteria.

Compliance with ethical standards

Acknowledgments

Our acknowledgments go to the participants that gave inform consent and took part in the study.

Disclosure of conflict of interest

No conflicts of interest.

Statement of ethical approval

The study was approval by the Health Research Ethics Committee of the Ambrose Alli University, Ekpoma (assigned number: 10/17 (001/17)).

Statement of informed consent

Informed consent form was duly signed and obtained from all study participants. The study was conducted following the WMA declaration of Helsinki-Ethical principles for medical research involving human subjects.
References


