



## Prevalence and antibiotic susceptibility pattern of agents of dental caries among patients in Uyo, Nigeria

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### Abstract

**Background:** Dental caries, a common oral disease globally affects nearly 100% of the population. Cariogenic bacteria implicated in caries are often resistant to many antibiotics' classes. This study was conducted to determine the prevalence and antibiotic susceptibility pattern of bacterial causes of dental caries among patients in Uyo, Akwa Ibom State, Nigeria.

**Methods:** One hundred and twenty (120) dental plaque samples were taken and inoculated into Basal salt medium and Basal salt medium agar plates separately. Different morphologically colonies of bacteria were isolated and identified through cultural, morphological and biochemical characteristics observed, according to Bergey's Manual of Systematic Bacteriology. Pure colonies (isolates) were biochemically confirmed with Vitek 2 System (bioMe'rieux), with their antibiotic susceptibility test.

**Results:** Culture growth of 27 (22.5%) bacterial isolates comprised mostly of *Burkholderia cepacia* group (*B. cepacia* complex) 7(5.8%). Others include *Coagulase Positive Staphylococcus* 3(2.5%), *Coagulase Negative Staphylococcus* 2(1.7%), *Enterococcus faecalis* 1(0.8%), *Enterococcus spp* (non *E. faecalis*) 1(0.8%), *Pediococcus pentosaceus* 1(0.8%), *Kocuria kristinae* 1(0.8%), *Proteus mirabilis* 4(3.3%), *Serratia ficaria* 2(1.7%), *Serratia marcescens* 2(1.7%), *Klebsiella pneumoniae* 1(0.8%), *Acinetobacter spp* 1(0.8%) and *Enterobacter cloacae ssp dissolvens* 1(0.8%). Among Gram-negative isolates, highest level of resistance was for Ceftazidime (66.7%) mostly attributed to *B. cepacia* isolates while high susceptibility to Levofloxacin was shown by Gram-positive isolates.

**Conclusions:** Detection of several species of bacteria that were not previously reported as caries aetiological bacteria, and high rate of antibiotic resistance exhibited by these agents of dental caries in Uyo is worrisome. This calls for regular antimicrobial surveys and infection control measures for improved treatment outcomes.

**Keywords:** Dental caries, prevalence, antibiotic resistance, fermentable-carbohydrates

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### Introduction

Dental caries is the gradual demineralisation of the tooth leading to cavity due to the activities of cariogenic bacteria on food debris over time.<sup>1</sup> It is known to be one of the most widespread persistent oral diseases globally, and one of the most preventable.<sup>2</sup> It is a multifactorial oral disease which occurs due to several interactions, such as; host oral cavity physiology, diet, fluoride, pH and the nature

of the tooth enamel, presence of cariogenic bacteria, over a long period of time.<sup>3</sup> It has been an established fact that the human mouth serves as the best habitat for vast bacterial species due to its alkaline condition, which is favourable to most microorganisms, and they are implicated in various oral diseases such as dental caries. The actual process that leads to dental caries begins with acidogenic and aciduric bacteria getting well established in the oral cavity, and producing biofilm on the tooth, in combination with other bacteria dwelling in the oral cavity.<sup>4,5</sup>

Bacterial plaque gets accumulated on dental surfaces, made up of oral normal flora, which is the primary bacteria aetiologic agent of caries. Cariogenic bacteria are capable of interacting through several ways such as; cell-cell communication, co-aggregation, metabolic exchange<sup>6,7</sup>, and exchange of genetic material.<sup>8</sup> The stated mechanisms ensure bacterial survival, as dental biofilms formed, makes therapeutic targets in dental diseases difficult. Dental caries can lead to destruction of enamel, dentin or cementum of teeth due to bacterial activities.<sup>9,10</sup>

The burden of dental caries is still reported to be a major health problem even in most industrialized countries.<sup>11</sup> This is largely due to the increasing consumption of sugar and inadequate exposure to fluorides.<sup>12</sup> It is known that when certain foods (especially carbohydrates) are eaten, the bacteria in the mouth (cariogenic bacteria) are capable of breaking them down to produce acids,<sup>13</sup> that have the ability to seriously damage the hard tissues of a previously healthy teeth. The result is the formation of dental caries (cavities).

Dental caries is more prevalent among adults than children. About 44 to 100% of adults are known to experience this oral disease worldwide, although in varying intensity.<sup>9,14,15,16</sup> About 49 to 83% of the entire populations across different countries are known to have dental caries.<sup>17</sup> The reduction in prevalence of dental caries worldwide was said to be as a result of the lower prevalence of the disease in children, which is documented to be 60-90%. Those who have poor oral hygiene and habits are one of the most affected people. Individuals living in poverty and minorities are affected more than their more affluent peers. Dental caries if left untreated, or not treated on time, can progress from stage 1 to 4, which eventually leads to stage 5, where the jaw becomes inflamed.

This is as a result of the pH in the biofilm constantly being below its critical level of 5.5 for enamel, and 6.2 for dentin for a long time. As the pH lowers, calcium and phosphate are gradually moved from the tooth to the biofilm to try establish an equilibrium. Hence, causing a net loss of minerals in the tooth referred to as demineralization. In scenarios that the pH in the biofilm gets to being neutral, the amount of soluble calcium and phosphate will be highly concentrated relative to that in the tooth, enabling minerals to be channelled back to partially demineralized enamel (remineralization). Therefore, it is mainly in situations whereby demineralization process persists more than remineralization process that cavity formation sets in and progresses. When caries is well established, it is usually challenging to treat because most of the bacteria aetiological agents of caries are resistant to commonly used antibiotics.<sup>18</sup> In a study conducted in Jos<sup>18</sup>, the antimicrobial sensitivity test of dental caries revealed that out of nine bacteria isolates, three were resistant to Vancomycin, one to Chloramphenicol, and four to Erythromycin. Of the observed isolates, *Enterobacter* species was resistant to all the three selected antibiotics while *Bacillus subtilis* and *Staphylococcus aureus* were resistant to Erythromycin and Vancomycin. In another study<sup>19</sup>, antimicrobial sensitivity of seven dental caries bacteria carried out, four were resistant to Vancomycin, Chloramphenicol, and Erythromycin. It was similarly reported by another researcher<sup>12</sup> that dental caries bacteria were resistant against Vancomycin, Chloramphenicol, Penicillin, Bacitracin and Streptomycin. This study was necessitated due to the paucity of data on the prevalence, as well as the antibiotic susceptibility pattern of bacterial causes of dental caries in Uyo, Akwa Ibom State, Nigeria.

## Materials and Methods

### Study design and Location

This research was a descriptive cross-sectional hospital-based study of 120 dental plaque samples from patients with dental caries attending the dental clinics at the University of Uyo Teaching Hospital (UUTH) and Saint Luke's Hospital, Anua both located in Uyo, the capital city of Akwa Ibom State, Nigeria.

**Study population**

This study involved 120 participants comprising male and female patients aged 3 to 72 years with dental caries attending tertiary and secondary health facilities in Uyo, Akwa Ibom State.

**Selection of Subjects: Inclusion/Exclusion Criteria****Inclusion Criteria**

Dental caries patients that had given their consent and were not on antibiotic treatment

**Exclusion Criteria**

Patients with dental caries and were on antibiotic treatment.

**Sample size determination**

This was calculated using the prevalence rate of 35.1% from a related study carried out in Port Harcourt, Rivers State, Nigeria.<sup>20</sup> The minimum sample size for this study was calculated using the formula developed by Godden<sup>21</sup> for sample size that is infinite, then the sample size was slightly reduced using Godden's formula for calculating sample size when population size is finite, to get the final sample size.

Calculation 1 (Infinite population – where population is greater than 50,000)

$$SS = Z^2 \times p \times (1-p) / C^2$$

$$SS = (1.9602)^2 \times 0.351 \times (1 - 0.351) / 0.05^2$$

$$SS = (3.842 \times 0.351 \times 0.649) / 0.003$$

$$SS = 291.733$$

$$SS \approx 292$$

Where:

SS = Sample size

Z = Z- value (1.96 or 1.960 for a 95% confidence interval)

$$Z^2 = 1.960^2 = 3.842$$

p = Percentage of population picking at choice, expressed as decimal

$$(p = 35.1\% = 35.1/100 = 0.351),$$

$$(1-p = 1 - 0.351 = 0.649)$$

C = Confidence interval, expressed as a decimal (0.05 confidence interval)

$$C = 0.05, C^2 = 0.05^2 = 0.0025 \quad C^2 \approx 0.003$$

Since the population of patients with dental caries attending health facilities in Uyo in the preceding year before the commencement of the study was 100 (less than the above sample size), Godden's

calculation for finite population was applied;

Calculation 2 (finite population – where population is less than 50,000)

$$SS = SS / 1 + (SS - 1) / Pop$$

$$SS = 292 / 1 + (292 - 1) / 100$$

$$SS = 292 / 1 + (291) / 100$$

$$SS = 292 / 3.91$$

$$SS = 74.68$$

$$SS \approx 75$$

Where:

SS = Final Sample Size

Pop = Population size or estimated population

SS = Sample Size derived from infinite population calculation.

Due to possibility of attrition of study participants, 10 % attrition rate was added to the minimum sample size. The minimum sample for the study was  $(10/100) \times 75 = 0.1 \times 75 = 7.5, \approx 8$ , therefore,  $75 + 8 = 83$  samples. Hence, the minimum sample size for this study = 80. However, a sample size of 120 was used.

**Sampling technique**

Participants for the study were recruited using Simple Random Sampling.

**Processing of samples**

Samples were collected by trained health professionals and processed at the Medical Microbiology Laboratory of the University of Uyo Teaching Hospital, Uyo. Swab samples from carious teeth of patients of which more than 70% of them had good oral hygiene (at the time of sample collection), were inoculated initially into Basal salt medium and Basal salt medium agar plates separately and Brain Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. To avoid contamination, only freshly aseptically prepared media were used (ensuring the use of face mask, sterile plates and other laboratory media preparation precaution for media contamination). The broth and plates that were inoculated were put into different sterile cellophanes and sealed using fresh laboratory masking tapes at all stages. This was followed by sub culturing them on

Blood agar (BA), MacConkey agar (MAC) and Cystine Lysine Electrolyte Deficient (CLED), and incubated at 37°C over night to yield colonies for isolates differentiation.<sup>22</sup>

Bacteria identification was done by observing; cultural, morphological and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology.<sup>23</sup> Gram staining of isolates were done and further identification using biochemical tests as well as antibiotic susceptibility tests were carried out using the Vitek 2 System (bioMe'rieux).<sup>24</sup>

### Interpretation of antibiotic susceptibility testing

Antibiotic susceptibility reactions of isolates were obtained within 12-18 hours of insertion of the antibiotic susceptibility test kits into the Vitek 2 System (bioMe'rieux) as; 'Resistant' 'Intermediate' or 'Susceptible', based on their turbidity level. In this study, the isolates that were resistant to at least one agent in three or more antimicrobial categories were regarded as multiple-drug-resistance (MDR).<sup>25</sup> Control strains were used to test the performance of the methods used and these included *Escherichia coli* ATCC 25922 for Gram-negative isolates and *Staphylococcus aureus* ATCC 25923 for Gram-positive isolates.<sup>26</sup>

### Statistical analysis

Statistical analysis was carried out using SPSS version 22.0 (Chicago, IL, USA). Associations between variables were considered statistically significant at p-values less than 0.05

Ethical Approval: Ethical approval was obtained from the ethical committees of UUTH and Akwa Ibom State Ministry of Health respectively

### Results

The 120 participants of this study consisted of persons between the ages of 3 to 72 years. The 33-42 years (40; 33.3%) had the highest number of participants. The female participants were more (70; 58.3%). Eighty-four (84; 70%), were people mostly in a marital relationship, while 80 participants (66.7%) were employed, and (90; 75%) were urban residents (Table 1). The cultured samples, yielded growth of 18 (15.0%) Gram-negative bacterial isolates of 7 different species, 9 (7.5%) yielded growth of Gram-positive bacterial isolates of 6 different species, while 93 (77.5%) yielded no

Table 1: Sociodemographic profile of study participants (n= 120)

| Variable                 | Frequency | Percent (%) |
|--------------------------|-----------|-------------|
| <b>Age (years)</b>       |           |             |
| 03 – 12                  | 6         | 5.0         |
| 13 – 22                  | 9         | 7.5         |
| 23 – 32                  | 15        | 12.5        |
| 33 – 42                  | 40        | 33.3        |
| 43 – 52                  | 30        | 25.0        |
| 53 – 62                  | 15        | 12.5        |
| 63 – 72                  | 5         | 4.2         |
| <b>Gender</b>            |           |             |
| Male                     | 50        | 41.7        |
| Female                   | 70        | 58.3        |
| <b>Marital Status</b>    |           |             |
| Married                  | 84        | 70.0        |
| Single                   | 36        | 30.0        |
| <b>Employment Status</b> |           |             |
| Employed                 | 80        | 66.7        |
| Unemployed               | 40        | 33.3        |
| <b>Residential Area</b>  |           |             |
| Urban                    | 90        | 75.0        |
| Rural                    | 30        | 25.0        |

Table 2: Antibiotic susceptibility of isolates using Vitek 2 System (bioMe'rieux) – Gram negative isolates

| Antibiotic                    | Resistant (%) | Intermediate (%) | Sensitive (%) |
|-------------------------------|---------------|------------------|---------------|
| Ampicillin                    | 8 (44.4)      | 6 (33.3)         | 4 (22.2)      |
| Ampicillin/Sulbactam          | 8 (44.4)      | 5 (27.8)         | 5 (27.8)      |
| Piperacillin                  | 9 (50.0)      | 6 (33.3)         | 3 (16.7)      |
| Cefazolin                     | 11 (61.1)     | 4 (22.2)         | 3 (16.7)      |
| Cefoxitin                     | 8 (44.4)      | 6 (33.3)         | 4 (22.2)      |
| Ceftazidime                   | 12 (66.7)     | 4 (22.2)         | 2 (11.1)      |
| Ceftriaxone                   | 7 (38.9)      | 8 (44.4)         | 3 (16.7)      |
| Cefepime                      | 11 (61.1)     | 4 (22.2)         | 3 (16.7)      |
| Ertapenem                     | 6 (33.3)      | 8 (44.4)         | 4 (22.2)      |
| Meropenem                     | 9 (50.0)      | 5 (27.8)         | 4 (22.2)      |
| Amikacin                      | 7 (38.9)      | 7 (38.9)         | 4 (22.2)      |
| Gentamicin                    | 6 (33.3)      | 7 (38.9)         | 5 (27.8)      |
| Tobramycin                    | 5 (27.8)      | 7 (38.9)         | 6 (33.3)      |
| Ciprofloxacin                 | 6 (33.3)      | 7 (38.9)         | 5 (27.8)      |
| Levofloxacin                  | 5 (27.8)      | 8 (44.4)         | 5 (27.8)      |
| Nitrofurantoin                | 5 (27.8)      | 7 (38.9)         | 6 (33.3)      |
| Trimethoprim/Sulfamethoxazole | 6 (33.3)      | 6 (33.3)         | 6 (33.3)      |

significant growth (Fig. 1).

The Antibiotic susceptibility pattern showed Gram-negative isolates being mostly sensitive to Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole, each having 6 isolates that were sensitive (33.3%), and mostly resistant to Ceftazidime 12 isolates (66.7%) as shown in Table 2, while Gram-positive isolates were mostly sensitive to Levofloxacin (7 isolates; 77.8%), and mostly resistant to Tetracycline, Quinupristin/Dalfopristin and Erythromycin with 77.8% each (Fig. 2).



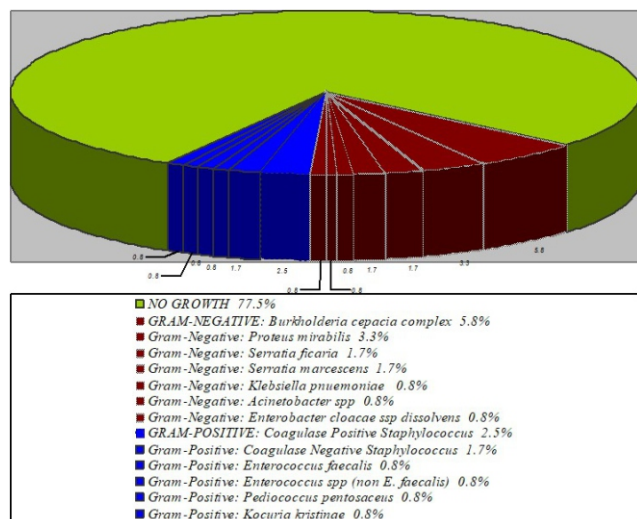


Figure 1: Distribution of species of organisms isolated

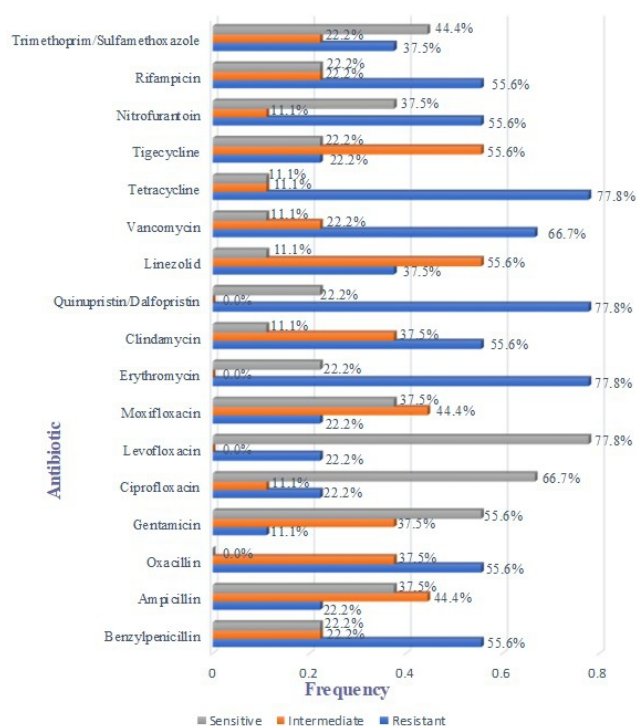


Figure 2: Antibiotic susceptibility of isolates using Vitek 2 System (bioMérieux) – Gram-positive isolates (n=9)

## Discussion

In this study, out of the one hundred and twenty dental plaque samples that were collected and processed, twenty-seven isolates at a prevalence rate of 22.5% were obtained. Whereas the result in a similar study carried out in Jos, Plateau State had a higher prevalence rate.<sup>18</sup> The study carried out in

Jos,<sup>18</sup> involved 150 dental plaque samples, with 95 isolates obtained, giving a prevalence rate of 63.3%. This indicates lower prevalence of dental caries in Uyo located at the Southern part of Nigeria in contrast to the higher prevalence rate recorded in Jos, located at the Northern part of Nigeria. The reason for the difference in prevalence rates in the two regions, may be due to differences in oral hygiene practice, and nutritional status or habits of the participants.

The various Gram-negative and Gram-positive isolates obtained from this study with *Burkholderia cepacia* complex 7 (5.8%) and *Coagulase Positive Staphylococcus* 3 (2.5%), as the highest respectively were indicative of opportunistic infection which is in conformity with reports from some researchers that the bacterial aetiology of dental caries are mainly by opportunistic oral flora. The species of bacteria implicated in dental caries and their numbers keeps changing often because of various modifying factors of caries.<sup>2,10,11,12,19,27</sup>

*S. mutans* was reported as the dominant bacteria aetiology of dental caries in similar previous studies.<sup>27,28</sup> In contrast, *Burkholderia cepacia* group (25.9%) also referred to as *Burkholderia cepacia* complex or *B. cepacia* or Bcc emerged as the dominant bacteria aetiology in this study.<sup>2</sup> Although in another study, it was reported that isolates such as *Streptococcus parasanguinis*, *Streptococcus mitis*, *Streptococcus oralis*, *Abiotrophia defectiva*, and *S. sanguinis* were the dominant bacterial flora of caries.<sup>29</sup> Other species were also reported in previous studies to be present in dental caries in higher numbers than *S. mutans* or even other bacterial species stated previously.<sup>18,30</sup> In one of such studies, *Lactobacillus* species was reported as the most prevalent isolate (28.4%), and *Fusobacterium* species (0.7%) as the least isolate.<sup>18</sup> This probably may be linked to the use of newer laboratory technique or the use of molecular diagnostic method(s) as was the case in this study. Whereas the dominant bacteria in this study *Burkholderia cepacia* has not been reported before in dental caries,<sup>2</sup> and it could have been taken to be *Pseudomonas* species if not for the newer laboratory method of identification used.

Even other less dominant isolates from this study such as *Proteus mirabilis* 4 (14.8%), *Coagulase Positive Staphylococcus* 3 (11.1%), *Coagulase Negative Staphylococcus* 2 (7.4%), *Serratia ficaria*

2 (7.4%), *Serratia marcescens* 2 (7.4%), *Enterococcus faecalis* 1 (3.7%), *Enterococcus* sp (non *E. faecalis*) 1 (3.7%), *Pediococcus pentosaceus* 1 (3.7%), *Kocuria kristinae* 1 (3.7%), *Klebsiella pneumoniae* 1 (3.7%), *Acinetobacter* spp 1 (3.7%) and *Enterobacter cloacae* ssp *dissolvens* 1 (3.7%), were rarely reported because of the routine isolation and identification methods used.<sup>2,10,11</sup> Nevertheless, these less dominant isolates were quite different from those reported by another researcher,<sup>30</sup> which included *Lactobacillus*, *Prevotella*, *Elenomonas*, *Dialister*, *Fusobacterium*, *Eubacterium*, *Olsenella*, *Bifidobacterium*, *Propionibacterium*, and *Pseudoramibacter*. Although *Staphylococcus* spp are present in the oral cavity of all orally healthy individuals, as it is a normal buccal cavity flora, it is only regarded as pathogens (opportunistic pathogens), when they are found in excess quantity than that found in a normal healthy teeth/mouth.

Antibiotic susceptibility pattern of isolates of this study showed all isolates being resistant to all the antibiotics used with varying degrees. The highest value of antibiotic resistance obtained for Gram-negative isolates were 66.7% for Cefotaxime (Table 2), which were mostly found amongst *B. cepacia* isolates, while Gram positive isolates recorded 77.8% for; Tetracycline, Quinupristin/Dalfopristin and Erythromycin each (Fig. 2), with *Enterococcus* spp being the only Gram-positive species recorded to be highly resistance to the three antibiotics that Gram-positive isolates showed highest resistance. The Gram-negative isolates were most sensitive to; Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole with 33.3% each (Table 2), with *Serratia ficaria* and *Proteus mirabilis* being the only isolates that were highly sensitive to at least two of the most sensitive Gram-negative antibiotics of this study, while Levofloxacin with 77.8% was the antibiotic that Gram-positive isolates were most sensitive to (Fig. 2), with *E. faecalis*, Coagulase positive *Staphylococcus* and Coagulase negative *Staphylococcus* having the highest levels of sensitivity.

In a similar study,<sup>18</sup> the antimicrobial sensitivity test carried out revealed that most bacterial isolates were more sensitive to the selected antibiotics used. Out of the nine bacterial isolates tested in their study, three were resistant to Vancomycin, one to Chloramphenicol, and four to Erythromycin, while *Enterobacter* species were the only species that were

resistant to all the three antibiotics used. On the other hand, *Bacillus subtilis* and *Staphylococcus aureus* were resistant to Erythromycin and Vancomycin. Whereas in another similar study,<sup>19</sup> four out of seven isolates obtained, were resistant to Vancomycin, Chloramphenicol, and Erythromycin. Similarly, in another study,<sup>12</sup> caries bacteria were resistant against Vancomycin, Chloramphenicol, Penicillin, Bacitracin and Streptomycin.

**Conclusion:** The prevalence of dental caries, the identification of several species of bacteria that were not previously isolated in caries, as well as the occurrence of *B. cepacia* complex as the dominant bacteria associated with caries among patients of the dental clinics of; UUTH and Saint Luke's Hospital-Anua, is worrisome. This is mostly due to the fact that the prevalence of this dominant bacteria seems to be higher than some of the documented cases. The bacteria were also seen to have the highest degree of antibiotic resistance, as such making the disease even more difficult to treat, and many of the respondents interviewed confided to have had other teeth affected within 5 months of treatment due to this challenge. Therefore, it is paramount for the government and other stakeholders in health care, to ensure prompt and effective management of bacterial infections to curtail antimicrobial resistance, help patients cope with the burden caused by the disease, as well as support researchers to undergo further studies to discover any other possible mechanism of antibiotic resistance present, this will help in prompt dental caries treatment or its eradication.

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