Original article

BACTERIOLOGY, PATHOLOGY AND ANTIBIOTIC SENSITIVITY OF PERIAPICAL INFECTIONS SEEN IN A TERTIARY HEALTH FACILITY

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ABSTRACT

BACKGROUND: Periapical infections present as symptomatic inflammatory reactions in the periapical tissues due to the presence of polymicrobial organisms and this may result in severe life-threatening infections. These lesions remain a public health concern.
AIM: This study aimed to identify the common bacteria involved in the periapical infections in our environment and to assess their susceptibility patterns to commonly used antibiotics, in the oral and maxillofacial out-patient clinic.
METHODS: Consecutive and consenting patients scheduled to have their teeth extracted by intra-alveolar protocol for reason of periapical infections and who claimed not to have taken antibiotics in the preceding one week were recruited into the study population. Nutrient agar, blood agar, chocolate agar and MacConkey agar were used for culture and antibiotic susceptibility tests. Organisms identified were subjected to various antibiotic susceptibility tests, of the commonly prescribed and used antibiotics (cefuroxime, erythromycin, gentamycin, ofloxacin, levofloxacin, amoxicillin and clavulanate, and obatrin) in the Dental Centre, using the disc diffusion method of Bauer and Kirby.
RESULTS: A total of eight hundred and ninety-eight (898) swabs were taken for culture, identification and sensitivity test from 530 females and 368 males aged 16-80years, presenting with periapical lesions. Of all the swabs taken, 135(15%) yielded no growths, 610(68%) yielded single organisms and 153(17%) yielded more than one organisms. Isolated bacterial organisms were *Staphylococcus albus* (22.4% of isolates), *Staphylococcus aureus* (50.0% of isolates), *Streptococcus* mutans (14.4% of isolates), *Streptococcus viridans* (13.0% of isolates) *and Klebsiella spp* (20.0% of isolates). The susceptibility rate of the tested antibiotics were amoxicillin and clavulanate 75%, cefuroxime 75%, obatrin 68%, ofloxacin 68%, erythromycin 62%, levofloxacin 59% and gentamycin 45%.
CONCLUSION: The study has provided evidence to show that facultative Gram positive cocci are the predominant organisms isolated from root apices with periapical lesions. Most of these infective organisms are susceptible to amoxicillin and clavulanate, and cefuroxime. They are resistance mainly to gentamycin.

Keywords: Periapical infections, culture, antibiotic susceptibility

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INTRODUCTION

Periapical infections usually result from a persistent inflammatory response induced by prolonged exposure of periapical tissues to various microbial agents, evoking an immunological reaction.[1] They involve polymicrobial infections that can be acute or chronic and give rise to a variety of clinical and radiographic manifestations. Once inflammation has spread from the dental pulp, it can produce a variety of apical pathologic changes; the most common are dentoalveolar abscesses, periapical granuloma and apical radicular cyst. Various factors such as the host resistance and the virulence of the bacteria affect the local inflammatory response in the periapical area often causing toothache.[2]

Periapical lesions of pulpal origin culminate from inflammatory response of the content of root canal system. An infection is produced if the invasion of microbes produces damage to tissues. Endodontic disease (pulpal and periradicular) is the result of both the pathogenic effects of the microbes and the response of the host. [3] The clash of the microbial and host defensive forces destroys much of the periapical tissues that results in the various types of periapical lesions which generally are barricaded in dense collagenous capsule. [4] These histological lesions comprise of acute and chronic inflammatory cells in variable concentrations and a wide variety of both Gram positive and Gram negative organisms. These lesions have epithelial strands that remain attached to extracted roots and these epithelial strands are believed to arise from rest cell of Malassez. [4]

Following pulp exposure as a result of caries, the microorganisms that initially occupy the pulp chamber and root canal lumen, invade the entire root canal system, that is, the dentinal tubules, lateral canals, accessory canals, secondary canals, apical delta ramifications, apical foramen and apical root cementum surface, [5,6] as well as invade the periapical tissues.[7] This infection leads to the development of apical periodontitis. Extraradicular infection is inaccessible to biomechanical root canal preparation and allows the persistence and multiplication of microorganisms.[8] Periapical granuloma which are chronic inflammatory lesions caused by complex polymicrobial are typified by damage to supporting periapical tissue including alveolar bone resorption around the apical areas and by granulomatous tissue with large numbers of inflammatory cells such as macrophages, lymphocytes, plasma cells and polymorphonuclear leukocytes. [9,10] Migration of inflammatory cells which predominantly express cytokines and growth factors that augment immune response seem to play an important role in the progression of periapical lesions.[11]

The most common pathway for microorganisms from the normal flora to the dental pulp is through an open cavity caused by dental caries.[12] Once the pulp is necrotic, dentinal tubules become dead tracts that microorganisms can traverse with impunity.[3] The necrotic pulp presents a polymicrobial flora characterized by a wide variety of combination of bacteria, averaging 4 to 7 species per canal, with approximately equal proportion of Gram positive and negative bacteria.[13]

A review of published studies have indicated that periapical lesions are rarely sterile and that associated microorganisms can be either opportunistic contamination because of oral communication [14] but, this infection remain a public health concern more so as the resultant pain causes unnecessary suffering, sleep disturbances and diminished productiveness and quality of life. Usual symptoms are pain, redness, swelling, heat and loss of function and these are precipitated as a response to bacterial presence and their products. Pain resulting from periapical lesions has remained the commonest reason of visit to the dentist. The periapical lesions are frequently underestimated in terms of morbidity and mortality. The risk of potential serious consequences arising from the spread from periapical lesion is still relevant today with many hospital admissions for dental sepsis. Treatment of these periapical lesions consists of removal of the source of infection by extirpation of the pulp, extracting the offending tooth with or without

incision and drainage as well as systemic antibiotics as an adjunct. A rational use of antibiotics implies that those with proven efficacy against common pathogen are employed in appropriate doses.

This study therefore is aimed at identifying the common bacteria involved in the periapical infections seen in our environment and to assess their susceptibility patterns to commonly used antibiotics, in the oral and maxillofacial out-patient clinic of the University of Benin Teaching Hospital, Benin-City, Nigeria with a view to providing appropriate antimicrobial agents which may be used to eliminate these pathogens, in an environment where self-medication has led to ineffective or failure of antibiotics treatment.

MATERIALS AND METHODS

This study was conducted over a period of 36 months (January 2010 to December 2012) at the Oral and Maxillofacial Surgery out-patient clinic of the University of Benin Teaching Hospital, a referral and tertiary care facility located in Benin City, Edo State, Nigeria. Benin City is a cosmopolitan City and capital of Edo State. This study protocol was approved by the Ethics committee of the University of Benin Teaching Hospital, Benin-City (Protocol No: ADM E 22/A/Vol. VII/835). All consecutive and consenting patients scheduled to have their teeth extracted by intra-alveolar protocol for reason of periapical infections and who claimed not to have taken antibiotics in the preceding one week were recruited into the study population. Patients were anesthetized with 1.8mls of 2% lignocaine hydrochloride and involved tooth extracted by intra-alveolar procedure. Immediately following extraction, samples were collected with a sterile swab stick and taken to the laboratory for culture using various agar plates.

The microbiological samples obtained from the extracted teeth were plated on chocolate agar (Oxoid No Cm 271), blood agar (Oxoid No Cm 271) and Mc Conkey agar (Oxoid No 7), while nutrient agar was used for susceptibility testing.[15] The agar were prepared according to the manufacturer's instruction and were incubated aerobically and anaerobically as the case may be. Samples inoculated on chocolate agar required 5 – 10 % carbon dioxide to create partial anaerobic conditions for microbial growth; and in this study the candle jar method at 37^0C was used to generate carbon dioxide.[15]

The various agar plates were streaked aseptically with sterile wire loop and well-spaced out to form discrete colonies. The inoculated plates were incubated at 37^0C. The plates which required anaerobic incubation were put in anaerobic jar filled with hydrogen gas, while others were incubated aerobically. Grease free slides were smeared with each specimen for Grams staining. The blood agar plate was incubated both aerobically and anaerobically. Plates were examined after 24 hours, and those which did not have growth were reincubated for another 24 hours. All the isolates were identified using colonial morphology and biochemical reactions (catalase, coagulase tube and slide tests as well as indole, citrate and urease tests) according to the methods of Cowan and Steel.[16]

Susceptibility test was done by the disc diffusion method of Bauer and Kirby.[17] With a sterile forceps, commercially prepared antibiotic discs were placed at least 25mm apart on nutrient agar plates for all isolates. The different antimicrobial agents used and their disc contents were: Cefuroxime (30µg), Erythromycin (30µg), Gentamycin (30µg), Ofloxacin (30µg), Obatrin (30µg), Levofloxacin (30µg), Amoxicillin and clavulanate (30µg). Plates were incubated at 37^0C for 24 hours, after which the zones of inhibition in each case were measured and compared to determine sensitive and non-sensitive organisms. "R" represents resistance, while "S" represents sensitivity to the antibiotic. Results were presented descriptively.

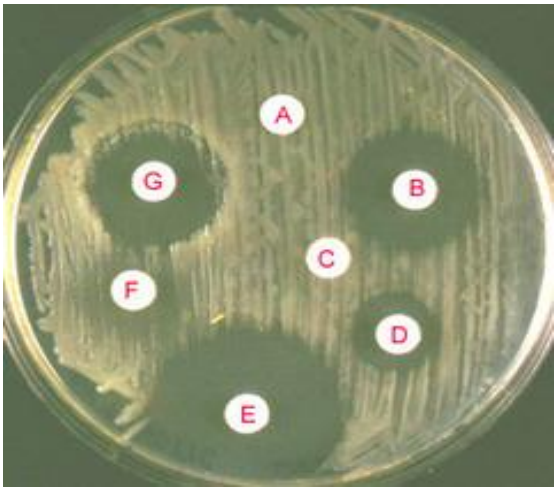

Figure 1: Demonstrating antibiotic sensitivity (B, E, G) and resistance (A, C, F).

RESULTS

A total of eight hundred and ninety-eight (898) samples were examined; 530 (59.0 %) were females while 368 (41.0%) were males, aged 16 – 80 years (Table 1; Figure 2).

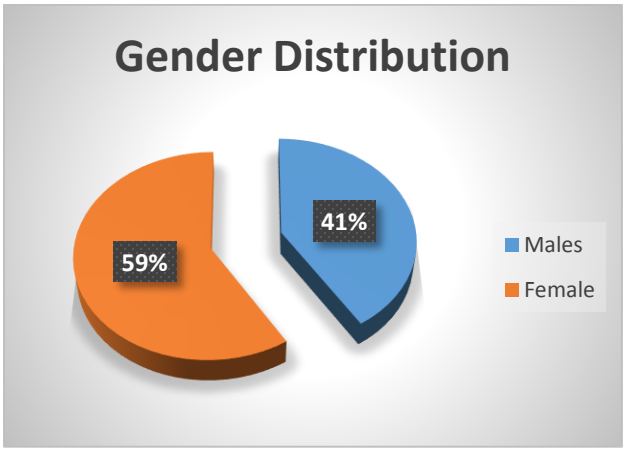

Figure 2: Gender distribution of patients recruited in the study.

Of all the swab samples examined, 763 (85.0%) were positive for microorganisms while 135 (15.0%) showed the absence of microorganisms. In about 17.0% (153) of the apices examined, infection was polymicrobial involving more than one microorganism (bacteria) while about 68.0% (610) were single infections (Figure 3).

Seven hundred and sixty-three (763) isolates belonging to five different species were recovered from the 898 root apices cultured after tooth extraction. All the bacteria isolates were facultative anaerobes in nature with Gram positive cocci being the most prevalent isolates accounting for over 95% of total isolates. Gram negative rods were recovered in about 5% of total isolates. *Staphylococcus aureus* was the predominant organism recovered accounting for 40.9% of total isolates. Other bacteria recovered from the root apices after culturing were *Streptococcus viridians*, *Streptococcus mutans*, *Staphylococcus albus* and *Klebsiella* sp. (Table 2).

The sensitivity of the isolates to selected antibiotics are shown in table 3; although the Streptococci and *Staphylococcus albus* were excluded from the sensitivity test since they are normal microflora of the mouth bearing the teeth. All the *Staphylococcus aureus* isolates showed a high degree of susceptibility to Augmentin and Cefuroxime and some degree of resistance to the other antibiotics, *Klebsiella* sp. was most susceptible to Cefuroxime and completely resistant to Gentamycin, Ofloxacin and Augmentin.

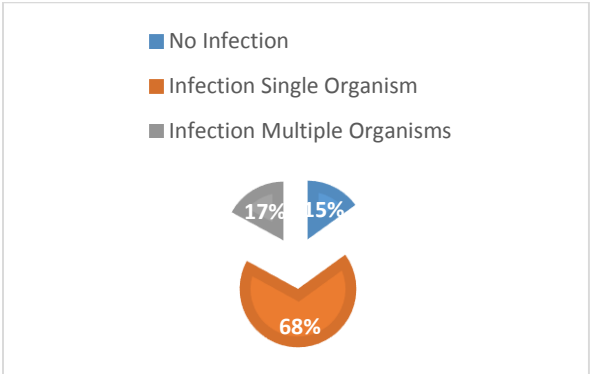

Figure 3: Distribution of bacterial infection on the root apices examined.

Table 1: Distribution of periapical lesions among different age ranges

Age Range (Years)	Diagnosis						
	AAP	**CAP**	**PAP**	**DAA**	**PAG**	**PAC**	**Total**
20 and less	112	16	34	57	9	2	**230**
21-30	91	21	21	26	5	4	**168**
31-40	74	20	23	17	3	5	**142**
41-50	60	15	18	9	7	1	**110**
51-60	48	12	7	16	9	6	**98**
61-70	34	15	9	11	9	5	**83**
71-80	16	13	9	12	11	6	**67**
Total	**435**	**112**	**121**	**148**	**53**	**29**	**898**

AAP- Acute apical periodontitis, CAP- Chronic apical periodontitis,
PAP- Periapical periodontitis

Table 2: Bacterial isolates recovered from periapical samples and their percentage occurrence

Bacterial Isolates	Frequency	Occurrence (%)
Staphylococcus albus	92	12.1
Staphylococcus aureus	312	40.9
Streptococcus mutans	110	14.4
Streptococcus viridians	96	12.6
Staphylococcus albus & *Klebsiella* sp.	79	10.4
Staphylococcus aureus & *Klebsiella* sp.	70	9.2
Streptococcus viridans & *Klebsiella* sp.	04	0.5

Table 3: Number and percentage of pathogenic bacterial isolates susceptible and resistant to selected antibiotics

ISOLATES	ANTIBIOTICS						
	CXM	E	CN	OFX	AUG	OB	LEV
Number of *Staphylococcus aureus* susceptible	234	194	140	213	235	213	185
% Susceptibility	75%	62%	45%	68%	75%	68%	59%
% Resistance	25%	38%	55%	32%	25%	32%	41%
Number of *Klebsiella* sp. susceptible	5	2	0	0	0	3	1
% Susceptibility	100%	40%	0%	0%	0%	60%	20%
% Resistance	0%	60%	100%	100%	100%	40%	80%

Total number of Staphylococcus aureus isolates = 312
Total number of Klebsiella sp. isolated = 5

KEY: CXM = Cefuroxime, E = Erythromycin, CN = Gentamycin, OFX = Ofloxacin, AUG = Augmentin (Amoxicillin & clavulanate), OB = Obatrin, LEV = Levofloxacin

DISCUSSION

Periapical lesions of endodontic origin are produced by an inflammatory response at the root apices of teeth with non-vital pulps.[18] Several studies[19,20] have demonstrated that microorganisms play an important role in the aetiology of pulpal and periapical pathosis in permanent teeth.[21]

In the present study, microorganisms were recovered from 763 (85.0%) of the 898 teeth examined. The findings that 14% of root canal had no culturable bacteria was not entirely unexpected. Earlier studies were unable to isolate bacteria from 55.6% [22] and 26.6%. [23] However, failure to detect bacteria does not prove their absence. It is possible that some microorganisms could have been lost, especially if the number of microrganisms in the root canal was very low or if they were present in inaccessible areas such as anatomical branches and apical areas obliterated by the previous treatment.[13] In this study, women were mostly involved with periapical lesions and this could be due to their love for sweetened food products which could be substrates for dental caries. It was also noticed that more females went for dental checkups because of their desires to take care of their bodies unlike the males. The highest frequency of lesions occurred between age ranges 20 years and below. This is because of the clear periodontium in youth which was necessary for the study to prevent contamination from the oral cavity.

This study clearly showed that *Staphylococcus aureus* is the predominant organism in swab specimens analyzed in our centre within the survey period. This situation could be as a result of the ubiquity of *S. aureus* as it colonizes body surfaces and also the invaginations of the nostrils, mouth, anus, vagina and urethra.[24] The colonization of these areas predisposes the individual to *S. aureus* infections.[25] Although some *Staphylococcus* strains are usually harmless as they are commensals, however, injury or break in the skin enables the organisms to invade the body and overcome the body's natural defenses. The consequences can range from minor lesions to deep-seated infections.[26] *S. aureus* is a hardy bacterium as it was shown in the study where it survived for three months on a piece of polyester, a material being the main material used in hospital privacy curtains.[27] The presence of Staph organisms on hospital equipment makes it rank as a nosocomial pathogen.[28] Some of the *S. aureus* isolated showed a clear zone of hemolysis around their colonies on blood agar identifying them as beta hemolytic *S. aureus.*

The pathogenic capacity of a given strain of *S. aureus* is the combined effect of extracellular factors and toxins together with the invasive property of the strain. Pathogenic invasive *S. aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic.[29] *Streptococcus mutans* was the next most isolated bacterium. It is a member of the Streptococcaceae and is usually found as a normal flora of the human oral cavity and upper respiratory tract. It is associated with the formation of dental caries.[30] A small percentage of this organism recovered were alpha hemolytic. *Streptococcus viridans* and *Staphylococcus albus* (a coagulase negative Staph) occurred almost at the same frequency; the both organisms are also natural flora of the oral cavity. *Klebsiella* spp were recovered from a small percentage of periapical swabs collected. This is one of the Gram negative organisms that are implicated in nosocomial infections. This organism a member of the Enterobacteriaceae is encapsulated with capsule serving as a virulence factor that shields the organism from antibiotics. Its clinical relevance lies in the fact that it is Gram negative which has the capability of producing extended spectrum betalactamase (ESBLs).[31] The ultimate goal of bacteria and other microbes is to reproduce and multiply; and for this reason, they spread to acquire uncolonized territories and new hosts.[32] Bacteria and other organisms colonize, reproduce and multiply in the pulp eventually migrating to the root apex, colonizing it causing necrosis.

The result of the present investigation indicate that tooth with periapical lesions, can habour pathogenic bacteria. This is consistent with all recent investigations and this implies that there exists a correlation between the presence of a

periapical radiolucency and the presence of microorganisms. The presence of facultative *Staphylococcus* and *Streptococcus* bacteria regarded as potentially virulent and invasive pathogens was a common finding in this study which is in line with the findings of Abou-Rass et al, [14] where the aforementioned organisms were common isolates when closed periapical lesions were sampled at the apex (53%). This study also identified Gram positive cocci as the most prevalent group of bacteria isolated, which is consistent with the findings of Cheung and Ho (2001).[33] They investigated the composition of microflora in endodontically treated teeth associated with asymptomatic periapical lesions in twenty-four southern Chinese patients. Facultative anaerobic bacteria were present in all, whereas strict anaerobic bacteria were found in few teeth with positive growth.

For antibiotic susceptibility, 75% of the pathogenic *S. aureus* which is the predominant bacterial isolate in this study were sensitive to Augmentin and were mostly resistant to Gentamycin. *Klebsiella* spp were resistant to a wide range of antibiotics especially Augmentin, Ofloxacin and Gentamycin. Antibiotic resistance arises because pathogens undergo evolutionary process, that is, natural selection which brings about alteration in phenotype and or genotype meaning the antibiotics can no longer target them.[34] This often arises because people abuse antibiotics. They stop when they are feeling better. There can be a few bacteria left over which develop resistance and proliferate faster than those which are still susceptible. Therefore resistance to antibiotics make them spread very fast. Unfortunately, many doctors prescribe antibiotics for complaints that could best be treated with aspirin and when an antibiotic is essential the effect is negative due to their excessive use that generates resistance. Too many pharmacies serve antibiotics across the counter and so many people are overdosing themselves. The more we use antibiotics and over use them, the more the microorganisms they kill develop defenses.[35]. The bacteriology of this study is not in consonance with most previous works in which strict anaerobes had always been the predominant organisms.

In conclusion, the study has provided evidence to show that facultative Gram positive cocci are the predominant organisms isolated from root apices with periapical lesions. Gram negative facultative rods are also isolated at low numbers. Virulence factors possessed by the pathogenic isolates (*S. aureus* and *Klebsiella* spp) have enabled some degree of resistance to some antibiotics; though a large percentage of pathogenic *S. aureus* were susceptible to Augmentin. In this study, 15% of patients presenting with periapical infections have the picture masked by antibiotic abuse or misuse. The findings of this investigation has isolated the commonest organism in periapical infection in our center and revealed their susceptibility patterns to commonly used antibiotics. Making the choice of Augmentin a prudent one for empirical therapy, before availability of microscopy, culture and susceptibility results.

Conflict of Interest: None declared

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