Microbial Analysis and Antimicrobial Susceptibility of Microorganisms Associated with Teeth Indicated for Non-Surgical Endodontic Treatment in a Nigerian Population--A Cross-Sectional Study

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ABSTRACT

Background

The success of endodontic therapy hinges on the eradication of microbial infection within the root canal system. Regional variations in microbial ecology and antibiotic resistance underscore the need for population-specific data to guide clinical protocols.

Objective

To characterize the microbial composition and antimicrobial susceptibility profiles of microorganisms isolated from root canals of teeth indicated for non-surgical endodontic treatment in a Nigerian cohort.

Methods

In this cross-sectional study, root canal samples were aseptically collected from 40 patients using sterile paper points after irrigation with saline. Specimens were cultured on Sabouraud dextrose, MacConkey, Nutrient, and Blood agar under aerobic and anaerobic conditions. Microbial identification was performed via biochemical assays, and antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method.

Results

Among 97 isolates, facultative anaerobes predominated (43.2%, n = 42), followed by fungi (38.2%, n = 37) and obligate aerobes (18.6%, n = 18). Staphylococcus spp. (44.4%) was the most prevalent aerobic bacteria, while Streptococcus spp. dominated both facultative (61.9%) and obligate anaerobic isolates. Yeasts constituted 64.9% of fungal isolates. All bacterial isolates exhibited resistance to co-trimoxazole. Resistance to ampicillin was universal among aerobes and anaerobes except Bacteroides spp. Aerobes demonstrated high sensitivity to gentamicin (100%), nalidixic acid (88.9%), and nitrofurantoin (94.4%), with exceptions observed in Klebsiella and Streptococcus spp. Anaerobes showed susceptibility to nitrofurantoin (95.2%) and gentamicin (90.5%), excluding Bacillus spp. Streptomycin resistance was widespread (95.2%) except in Pseudomonas spp.

Conclusion

Root canal infections in this population exhibit polymicrobial diversity with significant resistance to ampicillin and co-trimoxazole, rendering these agents ineffective. Gentamicin, nitrofurantoin, and nalidixic acid demonstrated superior efficacy, highlighting their potential utility in managing refractory infections. These findings advocate for antimicrobial stewardship and susceptibility-guided therapy in Nigerian endodontic practice.

INTRODUCTION

The preservation of teeth through endodontic treatment has gained prominence as patients increasingly opt to retain their natural dentition. A primary clinical objective is to deliver comprehensive treatment with successful outcomes, rooted in the elimination of infection and prevention of microbial invasion or reinfection of the root canal system and periradicular tissues.[1–2] Central to this goal is a thorough understanding of the endodontic microbiota associated with diverse disease presentations, which forms the foundation for effective therapeutic strategies.[3]

Scientific evidence unequivocally identifies microorganisms as pivotal drivers in the development, progression, and persistence of apical periodontitis.[2] Consequently, endodontic treatment aims to eradicate existing infections and establish a microbial barrier to prevent recurrence.[4] This underscores the necessity of characterizing the microbial profiles linked to different pathological

states, as such insights can inform the development of targeted preventive and therapeutic protocols.[3,5] Furthermore, cataloging endodontic bacteria may aid in identifying potential pathogens implicated in systemic diseases.[5] The dynamic evolution of these microbial communities, shaped by ecological shifts and interspecies interactions, further highlights the complexity of root canal infections.[4]

Despite advancements in understanding microbial diversity, the success rates of root canal treatments—particularly in teeth with necrotic pulps and periradicular lesions—have plateaued.[6,7] Current disinfection strategies combine mechanical instrumentation with adjunctive measures such as irrigants, intracanal medicaments, and antibiotics.[8] This study aimed to identify prevalent microorganisms in root canals of non-vital teeth requiring endodontic treatment and evaluate their antimicrobial susceptibility profiles.

METHODS

Study Design and Ethics

This cross-sectional study included 40 consecutive patients meeting predefined criteria, presenting for non-surgical root canal treatment at the University of Benin Teaching Hospital (UBTH). The study adhered to the STROBE guidelines and received ethical approval from UBTH's Research and Ethics Committee (ADM/E.22/A/VOL.VII/409). Written informed consent was obtained from all participants. The study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines and in adherence to the Declaration of Helsinki.

Inclusion Criteria

- Age between 16 and 60 years
- Non-mobile teeth with intact periodontal support

Exclusion Criteria

- Systemic diseases or immunocompromising conditions
- Teeth with swellings, sinuses, or periodontal pockets ≥4 mm
- Gingival recession >3 mm
- Prior root canal treatment or antibiotic use within one month
- Inability to achieve rubber dam isolation

Sampling and Clinical Procedures

Under aseptic conditions, teeth were cleaned with pumice, anesthetized using 2% lignocaine, and isolated with a rubber dam. Access cavities were prepared, and canals irrigated with sterile saline. Initial microbial samples were collected using sterile paper points placed 1 mm short of the apex. Post-instrumentation samples (following the use of Hedström files and 2.5% sodium hypochlorite) were collected 15 minutes later. Paper points were transferred to brain–heart infusion broth supplemented with 2% yeast extract.

Microbiological Analysis

Samples were vortexed, serially diluted (10^{-5}), and cultured on Sabouraud dextrose, MacConkey, nutrient, and blood agar. Aerobic cultures were incubated at 37°C for 24–48 hours, while anaerobic cultures were maintained in a gas chamber (37°C for 5 days). Isolates were identified via Gram staining and standard biochemical tests (oxidase, catalase, API, germ tube, urease), with comparison to standard reference strains.

Antimicrobial Susceptibility Testing

Susceptibility was assessed using the Kirby–Bauer disk diffusion method (Oxoid multidiscs). Inhibition zones were interpreted according to CLSI standards, using Staphylococcus aureus NCTC 6971 and Clostridium perfringens as control strains. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes.

Statistical Analysis

Data were analyzed using SPSS version 26.0. Descriptive statistics were used to summarize microbial prevalence and antimicrobial susceptibility. Chi-square tests were employed to compare observed versus expected frequencies of aerobic, anaerobic, and MDR isolates. Statistical significance was set at $p < 0.05$.

RESULTS

Organisms identified: All 40 samples (one per patient) yielded viable microbial cultures, with no exclusions. A total of 97 microorganisms were isolated, yielding an average of 2.5 organisms per sample. The microbial composition consisted of 43.2% obligate/facultative anaerobes, 38.2% fungi, and 18.6% aerobic bacteria (Figure 1). Anaerobes constituted the dominant group, followed by fungi, while aerobic bacteria represented the smallest proportion. Staphylococcal species were the most prevalent aerobic isolates, accounting for 44.4% of the aerobic bacteria.

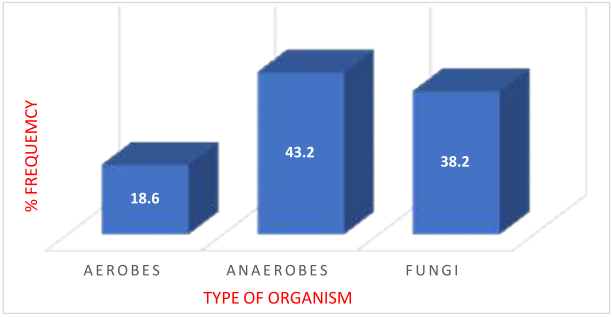

Figure 1: Frequency distribution of the microorganisms in the sample.

Among the anaerobes, facultative anaerobes comprised 61.9%, with Streptococcus species emerging as the most common facultative and obligate anaerobes. Among the fungi, yeast species—including Candida spp.—were the most frequently isolated. Notably, several biofilm-forming organisms were identified, including Streptococcus spp. (44.4% of aerobes), Staphylococcus spp. (44.4% of aerobes), and Candida spp. (within the 38.2% fungal isolates). These species are recognized for their role in the complexity of endodontic infections and their potential to mediate biofilm-associated antimicrobial resistance. (Table 1).

Table 1: *Distribution of root canal microorganisms*

Aerobes. 18 Isolates			Anaerobes. 42 Isolates						Fungi 37 Isolates		
			Facultative 26 Isolates			Obligates 16 isolates					
Organisms	No of Org.	% Freq. Distribution	Organisms	No of Org.	% Freq. Distribution	Organisms	No of Org.	% Freq. Distribution	Organisms	No of Org.	% Freq. Distribution
Streptococci spp.	2	3.33	*Streptococci spp.*	8	13.33	*Streptococci species*	5	8.33	*Mucor Species*	6	16.22
Straphylococcal spp	8	13.33	*Straphylococcal spp*	5	8.33	*Straphylococcal spp*	2	3.33	*Candida spp.*	7	18.92
E. coli	2	3.33	*E. coli*	6	10.00	*E. coli*	1	1.67	*Pencillium spp.*	4	10.81
Klebsiella spp.	2	3.33	*Pseudomonas spp.*	1	1.67	*Pseudomonas spp.*	1	1.67	*Yeast species*	9	24.32
Proteus spp.	1	1.67	*Fusobacterial spp.*	1	1.67	*Candida spp.*	2	3.33	*Microsporium spp*	2	5.41
Lactobacillus spp.	2	3.33	*Gram-ve rod spp.*	2	3.33	*Pseudomonas spp.*	4	6.67	*Fusarium spp.*	5	13.51
Bacillus spp.	1	1.67	*Branhamela*	2	3.33	*Bacteroides sp.*	1	1.67	*Blastomyces spp.*	1	2.70
			Calttarhalis spp.		1.67	*Fusobacteria sp.*			*Phycomyces spp.*	1	2.70
Brahamela spp.	-		*Bacillus spp.*	1					*Bacidiomyces spp*	2	5.41
	18			26			16			37	

Preponderance of Anaerobes: There was a significant discrepancy in the isolation frequencies of aerobic and anaerobic microorganisms. Aerobes were isolated 18 times, whereas anaerobes were isolated 42 times, a difference that was statistically significant (p = 0.00195) (Table 2).

Table 2: *Frequency of Aerobes vs Anaerobes*

Microbial Group	Observed Isolates	Expected Isolates	p-value	Significance
Aerobes	18	30	0.00195	Significant
Anaerobes	42	30		

Relative Prevalence of Organisms:

There was a statistically significant difference in the prevalence of microorganisms across the various groups (p = 0.0105). Fungi exhibited the highest individual group prevalence, while obligate anaerobes were the least frequently isolated. However, when grouped cumulatively, bacteria (aerobes and anaerobes combined) showed greater overall prevalence than fungi. Additionally, a comparison of observed versus expected frequencies revealed a non-uniform distribution, indicating that certain microbial groups were either more or less prevalent than would be expected by chance (Table 3).

Table 3: *Chi-square Test Results: Microbial Groups*

Microbial Group	Observed Isolates	Expected Isolates	p-value	Significance
Aerobes	18	24.25	0.0105	Significant
Facultative Anaerobes	26	24.25		
Obligate Anaerobes	16	24.25		
Fungi	37	24.25		

Multidrug Resistance (MDR) Assessment:

The antibiotics tested represented distinct antimicrobial classes, as outlined in Table 4. Based on the analysis, the multidrug resistance (MDR) rate was 85.7%, calculated by dividing the number of MDR isolates—defined as resistance to three or more antibiotic classes—by the total number of isolates tested. Specifically, 12 out of 14 isolates exhibited multidrug resistance.

Table 4: *The antibiotics tested and their antimicrobial classes*

Antibiotic	Class
AP (Ampicillin) 25 µg	β-lactam
SXT (Sulfamethoxazole/Trimethoprim) 25 µg	Sulfonamide
NA (Nalidixic Acid) 30 µ	Quinolone
S (Streptomycin) 25 µg	Aminoglycoside
F (Fusidic Acid) 200 µg	Fusidane
TE (Tetracycline) 50 µg	Tetracycline
GM (Gentamicin) 10 µg	Aminoglycoside

Other sensitivity findings in Antimicrobial Susceptibility Patterns:

All bacterial isolates exhibited resistance to co-trimoxazole, while all aerobes and anaerobes—except Bacteroides species—were resistant to ampicillin. Among the aerobes, all were sensitive to gentamicin, nalidixic acid, and nitrofurantoin, except Klebsiella species (resistant to nalidixic acid) and Streptococcus species (resistant to nitrofurantoin). Similarly, all anaerobes were sensitive to nitrofurantoin and gentamicin, except Bacillus species, which was resistant to gentamicin. Additionally, all anaerobic isolates were resistant to streptomycin, with the exception of Pseudomonas species.

Antimicrobial susceptibility testing revealed a concerning trend of widespread multidrug resistance (MDR) among aerobic organisms. Most tested species—including Staphylococci, Streptococci, E. coli, Klebsiella, Proteus, and Bacillus—demonstrated resistance to multiple antibiotic classes, meeting the criteria for MDR. Notably, Klebsiella species exhibited the broadest resistance profile, showing resistance to four distinct antibiotic classes. In contrast, Lactobacillus species was the only exception, displaying resistance to only two antibiotic classes and thus not classified as MDR (Table 5).

Table 5: *Antibiotic Resistance Patterns in Aerobic Organisms*

Bacterial Species	AP (β-lactam)	SXT (Sulfonamide)	NA (Quinolone)	S (Aminoglycoside)	F (Nitrofurantoin/Fusidane)	TE (Tetracycline)	GM (Aminoglycoside)	MDR (\u22653 Classes)?
Staphylococci spp.	R	R	S	S	S	R	S	Yes (AP, SXT, TE)
Streptococci spp.	R	R	S	S	R	S	S	Yes (AP, SXT, F)
E. coli species	R	R	S	R	S	R	S	Yes (AP, SXT, S)
Klebsiella spp.	R	R	R	R	S	S	S	Yes (AP, SXT, NA, S)
Proteus species	R	R	S	R	S	S	S	Yes (AP, SXT, S)
Bacillus species	R	R	S	R	S	R	S	Yes (AP, SXT, S)
Lactobacilli spp.	R	R	S	S	S	S	S	No (Resistant to 2 classes)

Antibiotic Resistance Patterns in Anaerobic Isolates

Antibiotic abbreviations: AP – Ampicillin; SXT – Co-trimoxazole; NA – Nalidixic acid; S – Streptomycin; F – Nitrofurantoin/Fusidane; TE – Tetracycline; GM – Gentamycin.

Antibiotic susceptibility testing revealed significant resistance patterns among anaerobic organisms. The tests were based on the multi-discs available at the time of the study and, while not exhaustive, included some antibiotics not routinely used in odontogenic infections, yet found to be effective in this context.

Testing against seven antimicrobial classes revealed that the majority of bacterial species—including Staphylococci, Streptococci, E. coli, Klebsiella, Proteus, and Bacillus—exhibited multidrug resistance (MDR). These organisms demonstrated resistance to multiple classes such as β-lactams, sulfonamides, quinolones, aminoglycosides, fusidane, and tetracyclines. Notably, Klebsiella species exhibited the broadest resistance profile, with resistance to four distinct antibiotic classes. In contrast, Lactobacillus species showed resistance to only two antimicrobial classes (β-lactams and sulfonamides) and thus did not meet the MDR criterion (Table 6).

Table 6: *Antibiotic Resistance Patterns in Anaerobic Organisms*

Bacterial Species	AP (β-lactam)	SXT (Sulfonamide)	NA (Quinolone)	S (Aminoglycoside)	F (Fusidane)	TE (Tetracycline)	GM (Aminoglycoside)	MDR (\u2265 3 Classes)?
Staphylococci spp.	R	R	R	R	S	S	S	Yes (AP, SXT, NA, S)
Streptococci spp.	R	R	S	R	S	S	S	Yes (AP, SXT, S)
E. coli species	R	R	S	R	S	S	S	Yes (AP, SXT, S)
Pseudomonas spp.	R	R	R	S	S	R	S	Yes (AP, SXT, NA)
Bacillus species	R	R	S	R	S	R	R	Yes (AP, SXT, S, TE)
Fusobacteria	R	R	R	R	S	S	S	Yes (AP, SXT, NA)
Bacteroides spp.	S	R	R	R	S	R	S	Yes (SXT, NA, TE)
β-haemolytic G-ve Rods	R	R	S	S	S	S	S	No (Resistant to 2 classes)

DISCUSSION

Findings:

The polymicrobial nature of root canal organisms is well established. Root canal diseases are triggered by polymicrobial infections, with the composition of these bacterial communities evolving over time and shaped by the dynamic root canal ecology and inter-species interactions.[4] The presentation of endodontic microorganisms in this study further underscores the polymicrobial nature of endodontic infections, as previously reported.[3] Bacteria were the predominant microorganisms isolated in this study, a finding consistent with previous reviews.[4,5] These results support the understanding that endodontic infections are associated with a wide range of microorganisms and that a single root canal system can harbor mixed flora.[16]

The relative prevalence of organisms in infected root canals remains of great interest to endodontists. Anaerobes—both obligate and facultative—were the most prevalent microorganisms recorded, with facultative anaerobes predominating. This contrasts with findings from a previous review article, which reported obligate anaerobes as the dominant microbiota in primary

infections.[3,16] In the early phases of root canal infection, the pulp contains higher oxygen tension and nutrient availability from the oral cavity, allowing facultative bacteria to thrive, resulting in increased bacterial counts and diversity.[17] Conversely, the carbohydrate-depleted yet protein-rich anaerobic environment typical of more established infections, particularly at the apical portions of the root canal, favors asaccharolytic obligate anaerobes.[18,19] This may account for the variation in anaerobic bacterial prevalence observed in this study compared to previous reviews.[3]

Streptococcus was the most frequently isolated anaerobe, a finding consistent with earlier reports that identified Streptococcus and Lactobacillus as predominant anaerobic isolates.[20] In contrast, Staphylococcal species were the most prevalent aerobes. Fungal species were also identified in this study, as previously reported.[4,21] However, while earlier literature emphasized Candida spp. as the most commonly isolated fungi,[4] our findings identified both Candida spp. and yeast spp. as prevalent, suggesting a broader spectrum of fungal presence. These findings emphasize the importance of microbiological testing prior to empirical antibiotic therapy, especially in recurrent or persistent root canal infections. Furthermore, they highlight the need to review endodontic disinfection protocols, given the significant presence of both facultative and obligate anaerobes.

Antimicrobial susceptibility testing revealed varied resistance and sensitivity patterns among the bacterial isolates. Notably, all isolates demonstrated resistance to co-trimoxazole and ampicillin, with the exception of Bacteroides spp., which was resistant only to ampicillin. This high level of resistance aligns with previous studies and underscores the limited utility of these antibiotics in root canal therapy. The pathogens identified—such as Streptococcus, Staphylococcus, E. coli, and Candida—are known biofilm formers. Biofilms, being complex microbial communities encased in an extracellular matrix, hinder antibiotic penetration and enhance resistance. Consequently, effective treatment requires a multifaceted approach incorporating mechanical debridement, antimicrobial irrigation (e.g., sodium hypochlorite or chlorhexidine), and adjunctive strategies like antimicrobial photodynamic therapy (aPDT) to disrupt biofilms.

Multidrug resistance (MDR) was a prominent feature in this study. The alarming MDR rate of 85.7% suggests pervasive resistance among both aerobic and anaerobic isolates. This may reflect the overuse of antibiotics in medical and dental practice, promoting selective pressure for resistant strains. Frequent resistance to β-lactams, sulfonamides, and aminoglycosides further indicates common prescription patterns contributing to resistance. These findings highlight the urgent need for antibiotic stewardship, emphasizing judicious antibiotic use and exploration of alternative treatment strategies to manage MDR among both aerobic and anaerobic organisms.

Aerobic isolates generally exhibited susceptibility to gentamicin, nalidixic acid, and nitrofurantoin. However, Klebsiella spp. and Streptococcus spp. were resistant to nalidixic acid and nitrofurantoin, respectively. The resistance pattern to nalidixic acid highlights the need for careful consideration when using this agent. Anaerobic isolates, with the exception of Pseudomonas spp., showed resistance to streptomycin. However, most anaerobes were susceptible to nitrofurantoin and gentamicin, except for Bacillus spp., which exhibited resistance to gentamicin.

The antibiotics assessed in this study were primarily antiseptics and bacteriostatic agents, as many common root canal antibiotics were unavailable for testing. Ampicillin (AP25 µg) and sulfamethoxazole-trimethoprim (SXT25 µg) exhibited universal resistance across all tested organisms, disqualifying them as reliable treatment options. Nalidixic acid (NA30 µg) and sulfonamides (S25 µg) also showed high resistance among key pathogens like E. coli, Klebsiella, Proteus, and Staphylococci spp., limiting their therapeutic potential. In contrast, fluoroquinolones (F200 µg) demonstrated consistent efficacy against both aerobic and anaerobic organisms, positioning them as robust options for managing persistent infections.

Tetracycline (TE50 µg) exhibited moderate effectiveness but was compromised by resistance, particularly among Staphylococci and Bacillus spp., reinforcing the importance of susceptibility testing. Gentamicin (GM10 µg) showed broad-spectrum efficacy, supporting its potential role in treating severe infections; however, its toxicity profile and dosing considerations must be carefully managed. These findings reaffirm the critical role of antimicrobial susceptibility testing in guiding antibiotic selection for root canal infections and underscore the need for continuous resistance surveillance to inform treatment decisions.

Implications: The antibiotics to which the organisms were most sensitive are primarily used for urinary tract infections and tend to concentrate within the urinary tract rather than the bloodstream. This pharmacokinetic limitation restricts their effectiveness in endodontic treatment when administered systemically. However, they may offer significant benefit when applied locally within the root canal system, suggesting a promising area for

further research into these potent yet underutilized antibiotics.

Given the high resistance rates to first-line antibiotics, fluoroquinolones (e.g., ciprofloxacin) or aminoglycosides (e.g., gentamicin) may be appropriate alternatives for empirical therapy. For mixed infections, combination therapy—such as metronidazole with ciprofloxacin—may offer superior outcomes due to broader anaerobic and aerobic coverage. Ampicillin and sulfonamides should be avoided as first-line options due to widespread resistance. Furthermore, the observed β-lactam resistance among Gram-negative rods suggests that amoxicillin monotherapy may be suboptimal. In persistent infections, comprehensive root canal disinfection is essential, especially in cases involving resistant Staphylococci or Bacteroides spp. The detection of fungal pathogens also calls attention to the potential need for incorporating antifungal agents in select cases.

Trade-Offs (Limitations):

This study's primary limitation lies in the reliance on conventional culture-based techniques for microbial identification, which may not fully capture the complexity of microbial dynamics. Future studies should incorporate advanced molecular methods to enhance detection and characterization.

Take-Home (Conclusion):

Non-vital root canal infections exhibit a polymicrobial profile, with Staphylococcal species being the most common aerobic isolate, Streptococcal species the most common anaerobe, and yeast species the most prevalent fungi. The microbial composition includes anaerobes (43.2%), fungi (38.2%), and aerobes (18.6%), each exhibiting diverse resistance mechanisms that influence treatment outcomes. The widespread resistance to ampicillin and co-trimoxazole limits their effectiveness in endodontic therapy.

Expectations for Future Research:

Traditional culture-dependent methods, while foundational, fail to account for many uncultivable microorganisms. Molecular techniques such as PCR and 16S rRNA sequencing can identify both cultivable and non-cultivable organisms, offering greater accuracy and insight into microbial ecosystems. Incorporating these advanced methods will provide a more nuanced understanding of root canal infections. Clinical guidelines should incorporate routine antibiotic susceptibility testing to inform targeted therapy. Empirical treatment strategies should prioritize fluoroquinolones and aminoglycosides, particularly in persistent or systemic infections. Combination therapies should be considered in mixed infections to enhance efficacy.

Recommendations

To address these challenges, the following actions are recommended:

1. Strengthen antibiotic stewardship through routine microbial culture and susceptibility testing, with a focus on narrow-spectrum antibiotics.
2. Refine empirical therapy guidelines to include metronidazole-based combinations for anaerobic coverage, alternative agents for resistant Gram-negative bacteria, and antifungal therapies where indicated.
3. Explore non-antibiotic therapies, such as antimicrobial photodynamic therapy and natural antimicrobial agents, to reduce reliance on antibiotics.
4. Conduct large-scale, multicenter studies to standardize treatment protocols, validate findings, and assess long-term clinical outcomes.

By translating these findings into evidence-based clinical practice, future research can enhance therapeutic effectiveness, minimize resistance development, and improve patient outcomes in endodontic infections.

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Conflicts of Interest:

The authors declare no conflicts of interest.

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