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Salivary Carriage of ESBL-Producing Coliforms in Root Canal Therapy Patients and Its Association with Antibiotic Use: A Brief Study

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Abstract

Background: *Saliva serves as a noninvasive medium for detecting extended-spectrum beta-lactamase (ESBL)-producing coliforms in patients undergoing root canal treatment (RCT). These bacteria have the potential to transfer antibiotic resistance genes to native oral biofilm microbes. Therefore, this study was done to check the presence of ESBL-producing coliform bacteria in the saliva of patients undergoing RCT and to explore their association with recent antibiotic use.*

Methods: *The study was conducted in a Dental College and Hospital located in the capital city of an eastern Indian state. Two millilitres of unstimulated saliva were collected from 20 patients undergoing RCT. Saliva samples were routinely processed using standard microbiological methods to identify bacteria, and the double-disk synergy test was used to confirm ESBL strains.*

Results: *Of the 20 saliva samples collected, 40% of the strains were identified as coliform bacteria. Furthermore, 3 of the 8 coliform isolates were identified as ESBL strains. ESBL colonisation showed a statistically significant association with prior antibiotic use (P-value=0.0079).*

Conclusion: *This pilot study suggests that coliform and ESBL-producing bacteria may colonise the oral cavity of patients undergoing endodontic treatment; however, the findings should be interpreted cautiously due to the small sample size. These results highlight the importance of strict antibiotic stewardship in dental settings to limit the emergence and spread of resistant strains.*

Keywords: Extended-spectrum beta-lactamase, Coliform bacteria, Root canal therapy, Antibiotic resistance, Oral microflora.

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Introduction

Coliform bacteria, particularly strains of *Escherichia coli* and *Klebsiella pneumoniae* that produce extended-spectrum beta-lactamases (ESBLs), are predominantly associated with the gastrointestinal tract. However, their presence has also been reported in the oral cavity under certain conditions, such as poor oral hygiene, immunosuppression, or recent antibiotic use. These organisms are often transient flora of the oral cavity, but their brief presence can still lead to dysbiosis[1]. These bacterial strains are capable of hydrolysing a broad range of beta-lactam antibiotics, including penicillin and third-generation cephalosporins, as well as many other antimicrobial agents, thereby making them multidrug-resistant (MDR). The rapid dissemination of ESBL genes, usually located on plasmids, exacerbates the public health risk[2].

Root canal treatment (RCT) is frequently accompanied by empirical antibiotic therapy, even when clinical indications are not strongly supportive. This practice, while sometimes unavoidable, can promote the emergence of antibiotic-resistant organisms. The use of broad-spectrum antibiotics in dental patients may facilitate the selection and colonisation of ESBL-producing bacteria within the oral cavity[3,4].

Saliva, as a representative medium of the oral ecosystem, can serve as an accessible and non-invasive sample for detecting such pathogens[5]. Despite growing global concern about antimicrobial resistance, limited research has focused on the oral cavity as a reservoir of ESBL-producing coliforms in dental patients. If not directly involved in oral microbial pathogens, these bacteria can transfer antibiotic-resistant genes to the normal oral microflora within the oral biofilm of these patients via various natural transfer mechanisms[5]. Therefore, this study was conducted to determine the presence of ESBL-producing coliforms in the saliva of patients undergoing RCT and assess their antimicrobial susceptibility patterns. Furthermore, the study sought to examine whether a correlation exists between recent antibiotic use and colonisation by these resistant strains.

Materials and Methods

This was a cross-sectional pilot study conducted in a Dental College and Hospital located in the capital city of an eastern Indian state. Due to logistical constraints, a formal sample size calculation was not performed. The study was conducted after Institutional Review Board approval. Twenty patients aged 18–50 years scheduled for RCT were enrolled based on predefined inclusion criteria, including no recent hospitalisation and no systemic illness. Patients receiving antibiotic therapy, using antiseptic mouthwashes, or with immunocompromised conditions were excluded. Detailed evaluation of oral hygiene status, periodontal condition, dietary habits, and socioeconomic factors was not performed. Although recent antibiotic use was recorded, information on antibiotic class, dosage, duration, and time since last use was not stratified. Differences in endodontic procedure stage were also not assessed and may represent potential confounding factors. 2 mL of unstimulated whole saliva from each patient

was collected in a sterile container before the beginning of their RCT. Under aseptic conditions, saliva samples were immediately transferred to the Central Research Laboratory for further processing[6]. The saliva samples were cultured on MacConkey agar and incubated at 37°C for 24 hours. Lactose-fermenting colonies were subjected to standard biochemical tests for bacterial identification[7]. Antimicrobial susceptibility of the isolates was tested by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, in accordance with the 2024 CLSI guidelines[7]. The antibiotics tested included ampicillin, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, and meropenem. Confirmation of ESBL production was performed using the double-disk synergy test (DDST) as previously described[8]. Statistical analysis was performed using SPSS version 25.0. Fisher's exact test was used to assess associations between categorical variables due to the small sample size. A P-value<0.05 was considered statistically significant. Results are interpreted with caution owing to limited statistical power.

Results

In this cross-sectional study of 20 patients undergoing root canal treatment, the mean age was 32.4 years, with a male predominance (60%). Recent antibiotic usage was reported in 30% (n=6) of the study population. Coliform bacteria were isolated from 8 out of 20 saliva samples (40%). Among these, *E. coli* was the most common isolate (n=5), followed by *K. pneumoniae* (n=3) (Table 1, Figure 1). ESBL production was confirmed in 3 of the 8 coliform isolates (37.5%), including two *E. coli* and one *K. pneumoniae* isolate. All ESBL-producing strains exhibited complete resistance (100%) to ampicillin, cefotaxime, and ceftazidime. One *E. coli* isolate was ciprofloxacin-resistant (50%), whereas all ESBL-positive isolates remained susceptible to gentamicin and meropenem (Figure 2). Notably, ESBL production was significantly associated with recent antibiotic use: all three ESBL-positive cases occurred among the six participants who had used antibiotics within the past four weeks (P-value=0.0079). These findings indicate a statistically significant association between recent antibiotic exposure and the presence of ESBL-producing organisms in the oral cavity; however, the strength and precision of this association are limited by the small sample size (Table 2, Figure 3).

Table 1. Demographic characteristics (n=20)

Parameter	Category	Frequency (n)	Percentage (%)
Gender	Male	12	60%
	Female	8	40%
Age (Mean±SD)	–	32.4±8.2 years	–
Recent antibiotic usage	Yes	6	30%
	No	14	70%

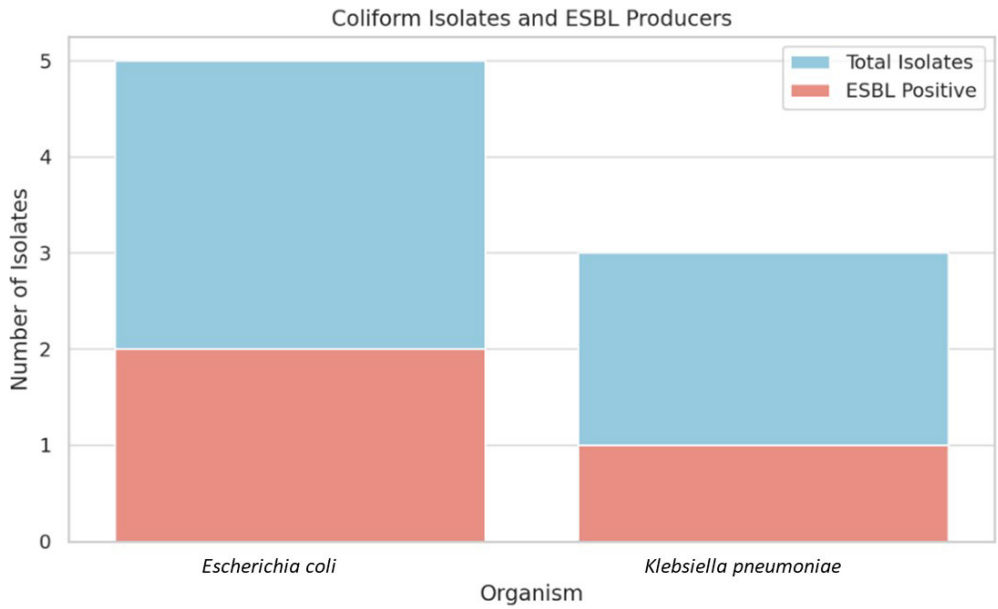

Figure 1. Coliform isolates and ESBL producers

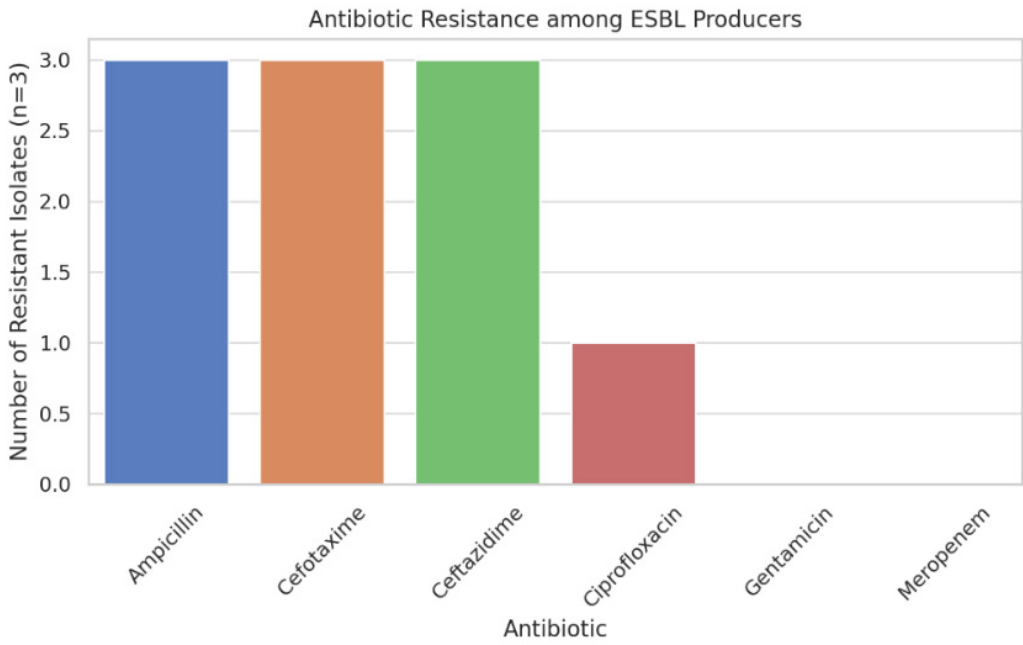

Figure 2. Antibiotic resistance among ESBL producers

Table 2. Overview of coliform prevalence, ESBL-producing organisms, antibiotic resistance, and association with recent antibiotic use (n=20)

Organism Isolated	Coliform Isolates (n)	ESBL Positive (n)	% ESBL Among Coliforms	Resistance						Recent Antibiotic Use (n)	ESBL +ve in Antibiotic Users	p-value
				Amp	Cef	Cft	Cip	Gen	Mer			
Escherichia coli	5	2	40.0%	2/2 (100%)	2/2 (100%)	2/2 (100%)	1/2 (50%)	0/2 (0%)	0/2 (0%)			
Klebsiella pneumoniae	3	1	33.3%	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	6	3	0.0079
Total (All Coliforms)	8	3	37.5%	3/3 (100%)	3/3 (100%)	3/3 (100%)	1/3 (33.3%)					

Note: Amp: Ampicillin; Cef: Cefotaxime; Cft: Ceftazidime; Cip: Ciprofloxacin; Gen: Gentamicin; Mer: Meropenem

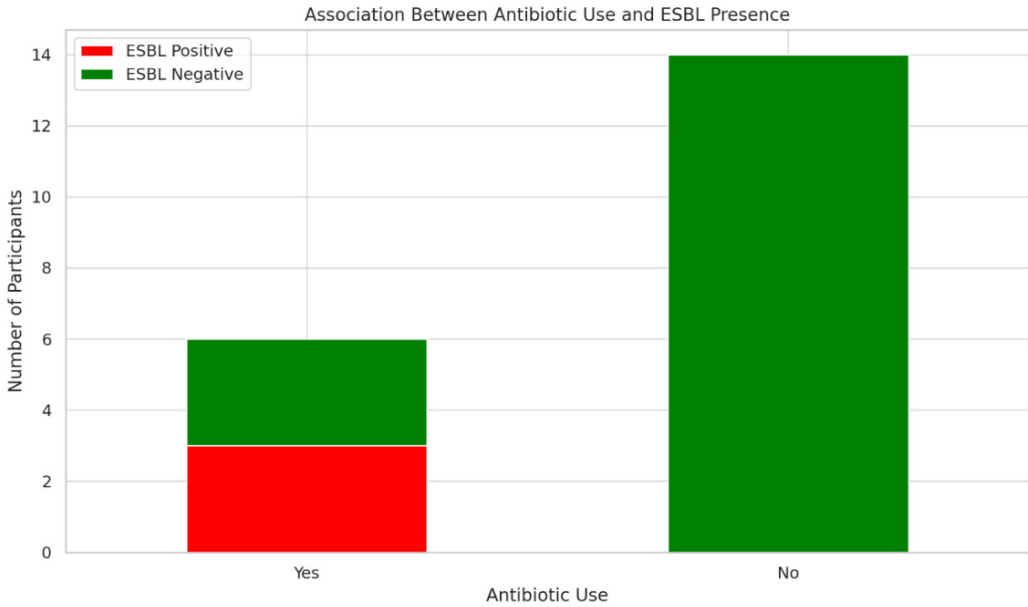

Figure 3. Association Between Antibiotic Use and ESBL Presence

Discussion

Traditionally, the oral cavity has never been associated with MDR gram-negative bacteria. This study indicates that ESBL-producing coliform bacteria can be present in the human oral cavity. Although the presence of these bacteria, particularly in patients with recent antibiotic exposure, is associated with RCT, this association underscores the role of empiric therapy in the emergence of MDR bacteria[3,4]. Although the number of participants in this study was limited, the findings are consistent with those from other studies worldwide: the prevalence of MDR bacteria is increasing among non-hospitalised individuals. The sample size is very small (n=20), which markedly limits the study's statistical power. As a result, the findings should be interpreted with caution, particularly with respect to estimating prevalence and drawing causal inferences. The small sample size also increases the likelihood of imprecision and reduces the generalizability of the results. When the oral cavity is subjected to antibiotic-induced dysbiosis, antibiotic resistance genes may be transferred to the oral microflora, leading to further systemic, untreatable oral infections. Moreover, aerosols generated during RCT increase the risk of environmental contamination and nosocomial transmission[9-11]. The antibiotic susceptibility results obtained in this study showed high resistance to third-generation cephalosporins and ampicillin. In contrast, they were sensitive to carbapenems and aminoglycosides, which is typical of ESBL-producing bacterial strains. This pattern again raises a question about the use of antibiotics in endodontics as empiric therapy[9-11].

Clinical Implications: The detection of ESBL-producing coliforms in saliva may represent transient colonisation resulting from antibiotic-induced ecological imbalance rather than stable oral residency. Dental procedures that disrupt oral biofilms and generate aerosols may further facilitate the short-term persistence and potential dissemination of these organisms. Although causal relationships cannot be established, these findings highlight concerns regarding the empirical prescribing of antibiotics in endodontic practice. The oral cavity may act as a temporary reservoir for antimicrobial resistance genes, underscoring the importance of strict antibiotic stewardship and adherence to evidence-based prescribing guidelines in dental settings.

Limitations: This study has several limitations. The small sample size significantly limits statistical power and generalizability. The cross-sectional design precludes causal inference. Molecular confirmation of ESBL genes was not performed. Oral hygiene status and detailed antibiotic exposure history were not quantified. Finally, the study was conducted at a single centre, which may not reflect broader population trends.

Conclusion: This pilot study identified ESBL-producing coliform bacteria in the saliva of patients undergoing root canal therapy, with a significant association with recent antibiotic use. This suggests that dental settings, especially those involving invasive procedures and empirical antibiotic administration, could contribute to the broader issue of antimicrobial resistance.

Ethical Considerations

The Institutional Review Board approved this study, vide letter number IRB/IDS/SOA-IIA dated 19 December 2024. Written consent from participants was obtained before the use of their samples.

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Conflict of Interest

None.

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