



PHENOTYPIC CHARACTERISTICS OF ENTEROCOCCUS FAECALIS ISOLATED FROM ENDODONTIC INFECTIONS

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ABSTRACT

Aim and objective: To evaluate the phenotypic characteristics of Enterococcus faecalis isolated from endodontic infections.

Background: Enterococcus faecalis is a microorganism detected in asymptomatic, persistent endodontic infections. E. faecalis has an ability to survive harsh environments including extreme alkaline pH. It resists bile salts, detergents, heavy metals, ethanol, azide & desiccation. E. faecalis endures prolonged period of nutritional deprivation. Enterococci can grow at 10 C and 45 C at pH 9.6 in 6.5% NaCl broth and survive at 60 C for 30 minutes. This may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments.

Materials and methods: A total of 20 samples were collected from patients undergoing endodontic treatment. The samples were inoculated in MacConkey agar and brain heart infusion agar. Presumptive identification of Enterococci was done by Gram's stain, Catalase test and Heat tolerance test in which Enterococci are gram positive cocci arranged in pairs. Brain-heart infusion agar (Oxoid) supplemented with 5% sheep blood was used for the detection of haemolytic activity. Gelatinase assay was carried out by adding an inoculum from a pure culture into tubes containing 12% gelatin in 0.8% nutrient broth.

Results: Cytolysin production was screened by cultivation on blood agar, 2(10%) isolates produced complete (β) haemolysis, 1(5%) isolate produced partial (α) haemolysis and the rest of the isolates did not produce haemolysis on blood agar (γ) haemolysis. Gelatinase assay by gelatin hydrolysis test revealed that 4(20%) of the isolates phenotypically expressed gelatinase gene as they could not liquefy gelatin media.

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INTRODUCTION

Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. They catabolize a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine, and many α keto acids(1). It is a member of the mammalian gastrointestinal microbiota but multidrug-resistant strains have been considered relevant causes of hospital-acquired and community related infections (2). Enterococci are common bacteria that inhabit the gastrointestinal tract, oral cavity, and vagina of humans and animals (3). Enterococcus faecalis is capable of surviving in a starved environment for a long period of time. It can invade the dentinal tubules and has the property of circumventing the antimicrobial effect of calcium hydroxide. It has several virulence factors such as aggregation substance, surface adhesions, gelatinase, and toxic cytolysin(4).

Enterococcus faecalis is often isolated from previously treated teeth with persistent diseases (5). Isolates from oral infections differ from clusters of hospital-derived isolates, as they do not present many mobile genetic elements. However, they usually carry virulence factors related to adhesion and biofilm formation, which may account for the colonization of different oral sites (2). The microenvironment of root canals may especially favour the survival of enterococci and the establishment of long-standing local infections. Other conditions, such as the quality of obturation, could conceivably also influence the colonization of E. faecalis and hence microflora in roots, either directly or indirectly (3). E. faecalis can adhere to root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm-producing organisms (6). The aim of this article is to evaluate the phenotypic characteristics of Enterococcus faecalis isolated from endodontic infections.

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MATERIALS AND METHODS

A total of 20 samples were collected from patients undergoing endodontic treatment. The samples were inoculated in MacConkey agar and brain heart infusion agar. Presumptive identification of Enterococci was done by Gram's stain, Catalase test and Heat tolerance test in which Enterococci are gram positive cocci arranged in pairs. Catalase negative, tolerance the temperature of 60°C for 30mins respectively. On MacConkey agar they showed small Lactose fermenting colonies.

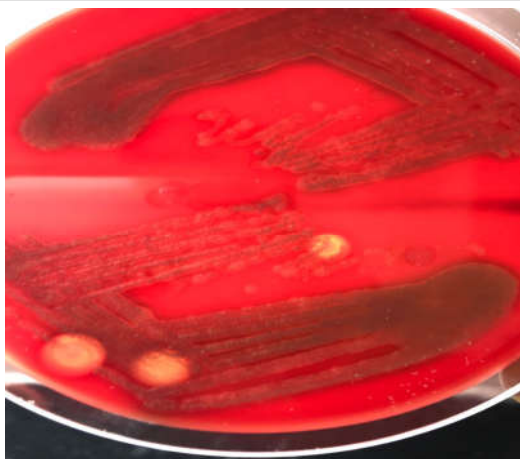
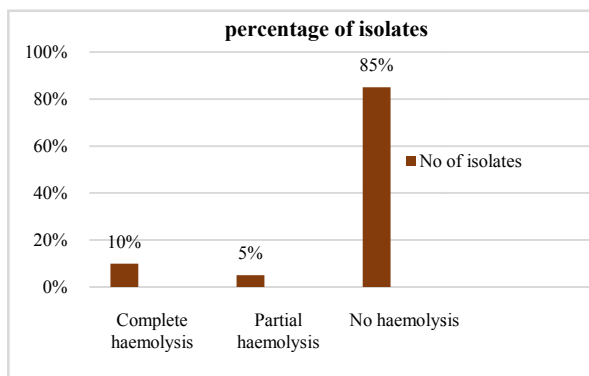
Detection of haemolytic activity and gelatinase activity

Brain-heart infusion agar (Oxoid) supplemented with 5% sheep blood was used for the detection of haemolytic activity. Pure isolates were cultivated on blood agar plates and plates were incubated at 37°C for 24h. Haemolytic activity was observed as (β) haemolysis surrounding bacterial colonies. (Complete haemolysis appeared as clear zones).

Gelatinase assay was carried out by adding an inoculum from a pure culture into tubes containing 12% gelatin in 0.8% nutrient broth. Tubes were incubated for 24-72 h at 37°C and then placed in the refrigerator for approximately 30 min. The liquefaction of gelatin was considered as positive result.

RESULTS AND DISCUSSION

Enterococcus faecalis possesses cytolysin or hemolysin as a virulence factor. Conflicting studies suggesting the role of cytolysin as a possible virulence factor. Initial studies reported that approximately 60% of *Enterococcus faecalis* isolates derived from fecal specimens from healthy individuals. However recent studies show that the role of cytolysin as a virulence factor is small or negligible (7).



Cytolysin production was screened by cultivation on blood agar, 2(10%) isolates produced complete (β) haemolysis, 1(5%) isolate produced partial (α) haemolysis and the rest of the isolates did not produce haemolysis on blood agar (γ) haemolysis.

Haemolytic activity

Enterococcus faecalis possess gelatinase which help it bind to gelatin and gelatinase levels were elevated in oral rises, crevicular fluid and whole saliva samples and in gingival biopsy specimens from periodontitis patients compared with healthy subjects. High gelatinase production has also been seen in epidemiologic studies with human clinical isolates (7).

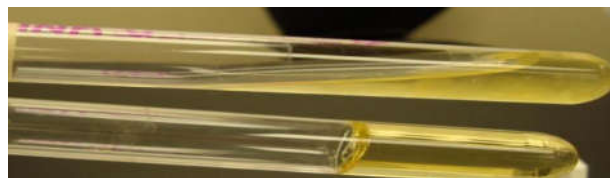
Gelatinase contributes to the bone resorption and degradation of dentin organic matrix, thus playing an important role in the pathogenesis of periapical inflammation.(8)

Gelatinase assay by gelatin hydrolysis test revealed that 4(20%) of the isolates phenotypically expressed gelatinase gene as they could not liquefy gelatin media.

Gustavo O. zoletti found all 20(100%) of strains presented the gelE gene (gelatinase). But, 10(50%) of them did not hydrolyse gelatin. Seven of the 10 gelatinase producing isolates were recovered from root canals with lesions, which suggests a role for its virulence factor in the pathogenesis of post treatment disease (9).

Renata Ximenes Lins et al found that all 20 isolates (100%) were positive for gelatinase (gelE) (10).

Gelatin liquefaction test



CONCLUSION

2(10%) isolates produced complete (β) haemolysis, 1(5%) isolate produced partial (α) haemolysis and the rest of the isolates did not produce haemolysis on blood agar (γ) haemolysis. 4(20%) of the isolates phenotypically expressed gelatinase gene. We can conclude that there may be a correlation between virulence factors detected and pathogenicity of *E. faecalis*. Further studies can be done to find the gene for haemolytic and gelatinase activity.

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