ABSTRAK

Enterococcus faecalis merupakan bakteri patogen oportunistik yang paling sering menyebabkan infeksi nosokomial pada manusia dan umum berbagai sampel klinis. Daya infektif *E. faecalis* yang tinggi mungkin terkait dengan kemampuannya menghasilkan banyak faktor virulensi yang sudah terbukti menginduksi infeksi pada manusia dan hewan coba. Akan tetapi, kontribusi keragaman fenotip dan genotip faktor virulensi tersebut pada kasus infeksi saluran akar gigi primer atau sekunder, khususnya yang terkait dengan kerusakan tulang periapikal, masih belum diketahui secara jelas sehingga masih sangat perlu dan relevan untuk dikaji. Penelitian ini dilakukan untuk menganalisis keterkaitan antara keragaman fenotip dan genotip faktor virulensi kapsul polisakarida (Cps), gelatinase (GelE) dan *adhesin of collagen for E. faecalis* (Ace) dengan sumber isolat (saliva dan saluran akar gigi nekrosis dengan atau tanpa kerusakan tulang periapikal) pada penderita infeksi saluran akar gigi primer.

Subjek adalah 18 individu dewasa laki-laki dan perempuan berusia 18-50 tahun yang menderita gigi nekrosis dan belum mendapatkan perawatan, yang terdiri atas 4 penderita dengan kerusakan tulang periapikal dan 14 penderita tanpa kerusakan tulang periapikal. Dari tiap subjek diambil sampel kerokan saluran akar gigi dan saliva yang selanjutnya dibagi dua. Satu bagian sampel diencerkan dalam NaCl 0.9% dan digunakan untuk pemeriksaan konsentrasi bakteri menggunakan *Real Time Polymerase Chain Reaction* (RT-PCR) menggunakan primer 16S rRNA *E. faecalis*. Satu bagian lagi dari sampel dibiakkan pada *ChromAgar plate* selama 18-24 jam (37°C) untuk identifikasi *Enterococcus* secara mikroskopis, biokimia (pewarnaan Gram dan uji katalase) dan molekuler dengan teknik PCR konvensional. Profil fenotip *E. faecalis* ditentukan secara mikrobiologi menggunakan *Trypticase Soy Agar* mengandung 1,5% *skim milk* (aktifitas GelE), secara molekuler menggunakan RT-PCR (ekspresi mRNA *ace*) dan secara biokimia menggunakan elektroforesis terhadap gen *cps* yang diamplifikasi dengan PCR (polimorfisme Cps). Keragaman genotip *E. faecalis* ditentukan berdasarkan analisis sekuen nukleotida gen *ace* dan *gelE* yang diperoleh menggunakan software 3730xl DNA Analyze dan Mega5. Data yang diperoleh dianalisis secara deskriptif.

Terdapat 19 isolat *E. faecalis* dari 36 koloni *Enterococcus* yang diisolasi saliva dan saluran akar gigi nekrosis penderita infeksi saluran akar primer dengan atau tanpa kerusakan tulang periapikal. Pada penderita tanpa kerusakan tulang, *E. faecalis* lebih sering ditemukan pada saliva daripada saluran akar gigi, sedangkan pada penderita dengan kerusakan tulang *E. faecalis* pada saliva dan saluran akar gigi ditemukan sama banyak. Pada kedua kelompok penderita *E. faecalis* terdapat sebagai infeksi campuran dengan bakteri lain. Konsentrasi *E. faecalis* terbesar (3.489 cfu/ml) dan terendah (2.117 cfu/ml) masing-masing ditemukan pada saluran akar gigi penderita dengan kerusakan tulang periapikal dan saluran akar gigi penderita tanpa kerusakan tulang periapikal. Sebagian besar (84,2%) isolat *E. faecalis* menunjukkan aktifitas gelatinase lemah (0.1-0.2 cm), sedang (0.3-0.8 cm), dan kuat (1-1.9 cm) serta hanya sedikit (15,8%) dengan aktifitas negatif.

Sebagian (8/19) isolat *E. faecalis* mengekspresikan gen *ace*. Isolat *E. faecalis* asal penderita dengan kerusakan tulang memiliki tingkat ekspresi mRNA ace yang lebih lemah (0,80) daripada isolat asal penderita tanpa kerusakan tulang (4,47). Sebagian besar (73,7%) isolat *E. faecalis* memiliki DNA Cps tipe 1, sedangkan sisanya memiliki DNA Cps tipe 2 (10,5%), tipe 5 (10,5%) atau tidak terdeteksi gen Cps (5,3%). Analisis sekuen gen *ace* dan *gelE* menunjukkan terdapatnya dua kelompok isolat *E. faecalis* dengan tingkat perbedaan masing-masing sebesar 0,6 dan 0,5 basa per 100 nukleotida. Isolat pada kluster 1 berada cabang filogenetik yang sama atau berdekatan dengan isolat *E. faecalis* ATCC 29212 kontrol dan isolat luar Indonesia (Amerika, Cina, India dan Portugal), sedangkan isolat kluster 2 berada pada cabang yang terpisah dari isolat tersebut.

Dari hasil konsentrasi *E. faecalis* pada saliva dan saluran akar gigi nekrosis penderita infeksi saluran akar primer dengan kerusakan tulang periapikal lebih tinggi daripada yang ditemukan pada saliva dan saluran akar gigi nekrosis penderita infeksi saluran akar primer tanpa kerusakan tulang periapikal. Tidak ditemukan kaitan antara kemampuan mengekspresikan kapsul polisakarida (Cps 2 dan 5), aktifitas gelatinase dan ekspresi mRNA ace dengan terjadinya kerusakan tulang periapikal pada penderita infeksi saluran akar gigi primer. Terdapatnya variasi genotip *gelE* dan *ace* isolat *E. faecalis* asal penderita infeksi saluran akar gigi primer di Aceh menunjukkan spesifisitas isolat ini dari isolat luar Indonesia, namun tidak ditemukan kaitannya dengan kejadian kerusakan tulang periapikal.

Kata Kunci: Enterococcus, Ace, gelE, sekuen, endodontik primer

ABSTRACT

Enterococcus faecalis is an opportunistic pathogenic bacterium that mostly causes nosocomial infection in human and commonly isolated in clinical samples. The highly infectious rate of *E. faecalis* might be related to its ability to produce a variety of virulence factors that have has been proved to induce infections in human and experimental animals. However, contribution of the phenotype and genotype profiles of virulence factors produced by *E. faecalis* to the case of primary or secondary endodontic infections had or had no periapical lesions is still not clearly understood so that it is important and relevant to investigate. This study is done to investigate the relationship between phenotype and genotype profile of *E. faecalis* with the sources of isolate i.e. root canal of necrotic teeth and saliva from patients suffered from primary endodontic infection had or had no periapical lesions.

Subjects were eighteen adult male and female individuals, aged 18-50 years old, suffered from primary endodontic infection. They consisted of 4 individuals had periapical lesions and 14 individuals had no periapical lesions, voluntarily participated in the study after completing the informed consent. Root canal scrapings and saliva were collected from each subject and diluted with 0.9% NaCl for further examination. Bacterial quantitation was done by Real Time Polymerase Chain Reaction (RT-PCR) using *E. faecalis* 16S rRNA primers. For isolation and identification, samples were cultured in ChromAgar medium, incubated at 37°C for 18-24 hours and observed using a binocular microscope. The pink colonies obtained were then identified using biochemical (Gram staining and catalase tests) and molecular biology (conventional PCR). Phenotype profile of *E. faecalis* was determined by microbiology method using *Trypticase Soy Agar* containing 1.5% *skim milk* (for GelE activity), by molecular biology approach using RT-PCR (for mRNA *ace* expression) and by biochemical method using agarose gel electrophoresis (for Cps polymorphism). Genotype profile of *E. faecalis* was determined based on the analysis of *gelE* and *ace* nucleotide sequences using web-based 3730xl DNA Analyze software. Data obtained was analyzed descriptively.

The results showed that bacterial isolation using ChromAgar medium followed by identification using both biochemical and molecular biology methods successfully obtained 19 *E. faecalis* isolates from 36 *Enterococcus* colonies isolated from root canals and saliva of patients suffered from primary endodontic infection had or had no periapical lesions. In the primary endodontic patient had no periapical lesions, *E. faecalis* isolates more frequently occurred in the saliva than in the root canals whereas similar prevalence of *E. faecalis* in saliva and root canals was found in the primary endodontic patients had periapical lesions. The highest (3.489 cfu/ml) and lowest (2.117 cfu/ml) proportions of *E. faecalis* were found in root canals of primary endodontic patients had periapical lesions and root canals of primary endodontic patients had no periapical lesions, respectively. The majority (84.2%) of *E. faecalis* isolates showed weak (0.1-0.2 cm), moderate (0.3-0.8 cm), and strong (1-1.9 cm) gelatinase activities and only some (15.8%) had no gelatinase activity. Some (8/19) *E. faecalis* isolates expressed mRNA *ace* that can

be categorized as weak, moderate or strong compared to the control *E. faecalis* ATCC 29212. *Enterococcus faecalis* isolated from primary endodontic patients had periapical lesions showed weak (0.80) mRNA ace expression ratio in comparison to that isolated from primary endodontic patients had no periapical lesions (4.47). The majority (73.7%) of *E. faecalis* isolates had type 1 of Cps DNA, and the rests had type 2 (10.5%), type 5 (10.5%) and no Cps gene (5.3%). Comparative nucleotide sequence analysis based on *ace* and *gelE* sequences indicated that *E. faecalis* strains isolated from the primary endodontic patients had or had no periapical lesions were grouped into two clusters with difference of less than 0.6 bases per 100 nucleotides. In the phylogenic tree, isolates in one cluster resided the same or closer branches to the control *E. faecalis* ATCC 29212 and overseas (America, China, India, South Korea, Japan and Portugal) isolates. Isolates in another cluster, on the other hand, resided a separated branch from these isolates in the phylogenic tree, indicating their unique and high specificity.

In conclusion, a simultaneously high proportion of *E. faecalis* in the root canals of saliva was related with the occurrence of periapical lesions in the patients suffered from primary endodontic infections, but not the variety of polysaccharide capsule (Cps) distribution, gelatinase (GelE) activity and mRNA *ace* expression levels. Polymorphism in the genotype of *gelE* and *ace* gene suggested the specificity some *E. faecalis* strains isolated from primary endodontic patients in Aceh from overseas isolates, but its strong relationship with the occurrence of periapical lesions was not found.

Keywords: Enterococcus, Ace, GelE, sequence, endodontic