

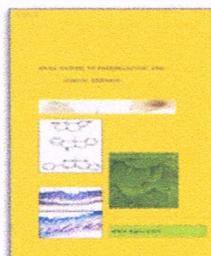
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IDENTIFICATION OF BACTERIA CAUSING NECROTIC PULP WITH 16S RRNA GENE POLYMERASE CHAIN REACTION AND ANTIBIOTIC RESISTANCE TESTING AT THE DENTAL HOSPITAL IN SEKELOA, BANDUNG, INDONESIA

Tina Rostinawati, Sulistyaningsih Hadisoebroto, Yoppi Iskandar, Prasetyo Hadi Nugroho, Audria Amanda Tara

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Nermeen Ashoush

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## IDENTIFICATION OF BACTERIA CAUSING NECROTIC PULP WITH 16S rRNA GENE POLYMERASE CHAIN REACTION AND ANTIBIOTIC RESISTANCE TESTING AT THE DENTAL HOSPITAL IN SEKELOA, BANDUNG, INDONESIA

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### ABSTRACT

**Objective:** This study aims to identify the bacteria that cause necrotic pulp in teeth of dental patients and test resistance of the bacteria found to antibiotics.

**Methods:** Bacteria were taken with paper points that were inserted into the root canal of patients at the Dental Hospital of the Faculty of Dentistry, Universitas Padjadjaran, Sekeloa, Bandung. Bacteria were cultured and Gram-stained. Bacterial DNA was isolated to be identified by polymerase chain reactions 16S rRNA method against known sequences of bacterial DNA. Then, identified bacteria were tested for antibiotic resistance to tetracycline (30 µg), clindamycin (2 µg), amoxicillin (10 µg), and ampicillin (10 µg).

**Results:** The 16s rRNA gene fragment of the main bacterium found was in 98% homology with 16S rRNA gene database in <http://blast.ncbi.nlm.nih.gov>, i.e., *Pseudomonas aeruginosa* and *Acinetobacter schindleri*. The results from inhibition zone of each antibiotic were 20.12 mm, 8.97 mm, 8.12 mm, and 8.03 mm for tetracycline, clindamycin, amoxicillin, and ampicillin, respectively, to *P. aeruginosa*. While inhibition zone of tetracycline to *A. schindleri* was 37.7 mm.

**Conclusion:** Based on the study results, *P. aeruginosa* from patients with necrotic pulp samples were resistant to clindamycin, amoxicillin, ampicillin, and decreased activity to tetracycline. While *Acinetobacter schindleri* was still sensitive to tetracycline.

**Keywords:** Identification of bacteria, Necrotic pulp, Polymerase chain reaction of 16S rRNA gene, Sequencing, Antibiotic resistance test.

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### INTRODUCTION

The necrotic pulp is found in a non-vital tooth that is damaged throughout the pulp (root canals), which is generally caused by microorganisms that cause cavities. Inflammatory damage will occur if the microorganism cause symptoms and clinical signs in the host. Bacteria are a major causal factor in the development of necrotic pulp and periapical disease [1]. Microorganisms get into the pulp in several ways, the most common is an open pit cavity due to dental caries. In addition, microorganisms can reach the pulp due to trauma or mechanical injury, gum sulcus, and through the bloodstream [1]. Based on data from the Household Health Survey (Household) in 2004 by the Ministry of Health of the Republic of Indonesia, the prevalence of dental caries in the population is 90.05% [2]. Pulp and periapical diseases are caused by several types of bacteria, especially anaerobic bacteria, such as *Osenellauli*, *Eikenellacorrodens*, *Porphyromonas endodontalis*, *Peptostreptococcus*, *Bakterioides*, *Eubacterium*, *Staphylococcus aureus*, and *Fusobacterium nucleatum* [3]. One treatment of necrotic pulp includes root canal irrigation with antibiotics. The solution contained the antibiotic is used to dissolve the debris resulting from root canal preparation and also to disinfect the root canal. Irrigation solution commonly used in dentistry included the class of antibiotics such as mixture of tetracycline and disinfectants and tetraclean, containing a mixture of doxycycline 500 mg/ml with propylene glycol [4,5]. The ability of the irrigation solution to kill bacteria had become more limited, concomitant with the discovery of genes encoding tetracycline resistance in mouth bacteria, i.e., tet (M), tet (O), tet (Q), tet (W), tet (32), and tet (A) [6]. A large number of patients with necrotic pulp were recorded, around 122,467 and a total of 234,083 cases of complaints of oral disease in Indonesia. This indicated that recurrent therapeutic treatment is required and a possibility of

antibiotic resistance in bacteria that cause necrotic pulp [7]. The fact that high rates of necrotic pulp are necessary to attempt the detection of bacteria causing the infection and the sensitivity to antibiotics that have not been reported so far in Indonesia's Dental Hospital. This study was conducted to determine the types of microorganisms that cause necrotic pulp and resistance patterns of these microorganisms to antibiotics for therapeutic effectiveness.

### METHODS

The Ethical Committee approval was provided by Medical Faculty of Universitas Padjadjaran in Indonesia, Code No:041/UN6.C1.3.2/KEPK/PN/2016.

#### Sampling bacteria in necrotic pulp

Samples were taken by one of the Hospital Dental Faculty of Dentistry, University of Padjadjaran, Sekeloa, Bandung. Samples were taken from the plaque of patients with necrotic pulp using sterilized equipment. Plaque samples were then inserted into a transport medium containing sterilized trypticase soy broth (TSB). The samples were then taken to the Laboratory of Microbiology, Faculty of Pharmacy, University of Padjadjaran, and incubated at 37°C for 18-24 hrs.

#### Isolation of bacteria causing necrotic pulp

A suspension of the incubated bacteria was then transferred to trypticase in soy agar (TSA) with the incised plate method. A total of 30 ml of sterile TSA media was poured into a Petri dish and then allowed to solidify. A total of 1 loopful bacterial suspension taken from TSB medium and then smeared onto TSA media that had been overbooked.