

THE IMPACT OF EXPOSURE TO ELECTRIC AND TOBACCO CIGARETTE SMOKE ON THE GROWTH OF *STREPTOCOCCUS PNEUMONIAE*, *KLEBSIELLA PNEUMONIAE* AND *MYCOBACTERIUM TUBERCULOSIS* IN VITRO, A PRELIMINARY STUDY

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Abstract – Consumption of electric cigarettes (e-cigar) in various countries continues to increase every year. The results of research on the impact of exposure to e-cigar smoke produce varied information. The prevalence and severity of *Pneumonia* and *Tuberculosis* are influenced by smoking of tobacco cigarettes (t-cigar). This study aims to test the effect of e-cigar and t-cigar smoke exposure on the growth of *S. pneumoniae*, *K. pneumoniae* and *M. tuberculosis* in vitro. A descriptive experiment was conducted using three different types of bacteria. The treatment was exposure of e-cigar and t-cigar smoke, as well as no exposure of the both smoke as a control. Power of inhibition e-cigar and t-cigar were calculated using Poisoned food method. The growth of *S. pneumoniae* is more responsive to exposure to cigarette smoke (e-cigar and t-cigar). In the control group, *S. pneumoniae* colonies were smaller than *K. pneumoniae*. E-cigar and t-cigar smoke inhibit the growth of *S. pneumoniae* and *K. pneumoniae*, but e-cigar smoke showed more significant inhibition than t-cigar smoke. Further in vivo research is still needed to analyse the impact of e-cigar smoke exposure on human health.

INTRODUCTION

The trend of the use of electronic cigarettes (e-cigar) in various countries has been increasing lately. The user of e-cigar expects healthier smoke than tobacco cigarettes (t-cigar). In the USA, the e-cigar user increase from 1.8% in 2010 to 13.0% in 2013 (McMillen *et al.*, 2015). While in the UK, the e-cigar user increase from 7% in 2016 to 11% in 2017 (Bauld *et al.*, 2017). Russia and Lithuania are the two countries with the highest e-cigar user in Europa (Brozek *et al.*, 2018). The study in four countries (Indonesia, Malaysia, Qatar and Greece) showed that an estimated 818,500 people are currently using the e-cigar. Among the e-cigar users, 64.4% and 84.1% are also smoke t-cigar in Greece and Qatar, respectively. On the other hand, among the t-cigar users, 10.6 and 6.0% were not smoking e-cigar in Greece and Qatar, respectively (Palipudi *et al.*, 2016).

Compared to t-cigar smoke, the composition of e-

cigar smoke is dominated by synthetic substances, *e.i.* Carbonyl compounds (acetaldehyde, acrolein, formaldehyde, methyl benzaldehyde), volatile organic compounds (toluene and xylene), tobacco-specific nitrosamines, and metals (candium, lead and nickel) (Goniewicz *et al.*, 2013), nicotine, cotinine, propylene glycol, acetone, glycerin, methyl butyl-methyl butanoate, chromium (Qasim *et al.*, 2017).

The benefit of e-cigar instead of t-cigar is still a hot topic of discussion among researchers and produce diverse scientific information. However, some reports showed that there is no significant difference between the two types of exposure of smoke cigars on oral or gut microbial communities (Stewart *et al.*, 2018), nicotinerig impact (Flouris *et al.*, 2013), toxicity in bronchial epithelial cells (BEC) and Calu-3 cells (Higham *et al.*, 2018). On the other hand, some reports showing the differential effect between e-cigar and t-cigar smoke on bacterial

growth. T-cigar smoke exposure dramatically decreased bacterial survival and growth, while flavourless e-cigar aerosol has only a small effect, and flavourless aerosol containing nicotine has a modest effect on the bacterial growth (Cuadra *et al.*, 2019).

In developing countries, pneumonia and lung tuberculosis is still a global health problem. The incidence of pneumonia is estimated to be around 0.29 events per year. The highest incidence rates occurred in India (6 million each), China (21 million each) and Pakistan (10 million each) and other countries such as Bangladesh, Indonesia and Nigeria (6 million each). Pneumonia is considered responsible for 19% of all deaths of a child under five years, which is more than 70% occurring in Sub-Saharan Africa and Southeast Asia (Ramezani *et al.*, 2015; Rudan *et al.*, 2008). Globally in 2016, there were 10.4 million cases of tuberculosis (TB) incidents. Five countries with the highest incidence of cases are India, Indonesia, China, Philippines and Pakistan. Most of the estimated TB incidents in 2016 occurred in the Southeast Asia Region (45%) where Indonesia is one of them, and 25% occurred in Africa. Until 2017 in Indonesia, 420,994 TB cases were positive (Indonesian Ministry of Health, 2018). A systematic review and meta-analysis, and previous research in South Korea showed that smoking increases the risk of TB and pneumonia (Baskaran *et al.*, 2019; Jee *et al.*, 2009). To enrich information regarding e-cigar smoke against pathogenic bacteria, here we describe the differential effect of e-cigar and t-cigar smoke exposure on the growth of common bacterial pathogens responsible for typical pneumonia and tuberculosis TB (*Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis*) (Bedi, 2006; Chai *et al.*, 2018).

MATERIALS AND METHODS

Reagents and Bacterial Strains

All bacteria (*S. pneumoniae*, *K. pneumoniae* and *M. tuberculosis*) were obtained from the Biological Molecular and Immunology Laboratory, Faculty of Medicine, Hasanudin University, Indonesia. Merck and Hardy Diagnostik, respectively provided Mac Conkey Agar (MCA) and Lowenstein Jensen Agar (LJA). E-cigar liquid composed of grade vegetable glycerin, usp propylene glycol, organic flavouring, water and nicotine. T-cigar composed of 31 mg of tar, 2.2 mg of nicotine per 1 g.

Experimental design and data analysis

A descriptive experiment (exposure of cigarette smoke) was conducted using three different types of bacteria (*Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis*). The treatment was exposure of e-cigar and t-cigar smoke, and control (no exposure of cigarette smoke). Power of inhibition of e-cigar and t-cigar smoke on the three bacteria were calculated using Poisoned food method (Balouiri *et al.*, 2016). Inhibition power (%) = $((D_c - D_s) / D_c) \times 100$, where D_c is the diameter of growth in control plate and D_s is the diameter of growth in the plate exposure by cigarette smoke.

Experimental procedure

Three different plates were provided for *S. pneumoniae* and *K. pneumoniae* (MCA), as well as *M. tuberculosis* (LJA). The media in the plates were exposed in a sterile condition by e-cigar and t-cigar smoke every day for 8 days except the LJA media, which was exposed more than 5 weeks. The e-cigar or t-cigar smoke were sucked through a syringe (150 mL) equipped with a hose, and the smoke was exhaled to the surface of media. After the concentration of the smoke in the plate decreased, then the smoke exhalation was repeated until it consumed one cigarette stick (smoking spend of 7 minutes). The exposure media were then incubated at 37 °C. The diameter of the bacterial colony was observed and measured by digital callipers carbon fibre (0.0 mm) (Monotaro, Japan) following 24 hours of incubation. This treatment was repeated for up to 8 days, except for *M. tuberculosis* culture, which treated more than 5 weeks to see the growth of the bacteria.

RESULTS AND DISCUSSION

The effect of cigarettes smoke on the growth of three pathogenic bacteria

The growth of *S. pneumoniae* and *K. pneumoniae* on the MCA could be observed following 24 h of incubation. With the condition of smoke exposure since one day after incubation, the *S. pneumoniae* grew faster and reached the stationer phase faster (± 3 days of incubation) than *K. pneumoniae* (± 7 days of incubation). However, *M. tuberculosis* grew very slowly. The colony of *M. tuberculosis* could not be detected until 5 weeks of incubation (with the condition of smoke exposure at each day of

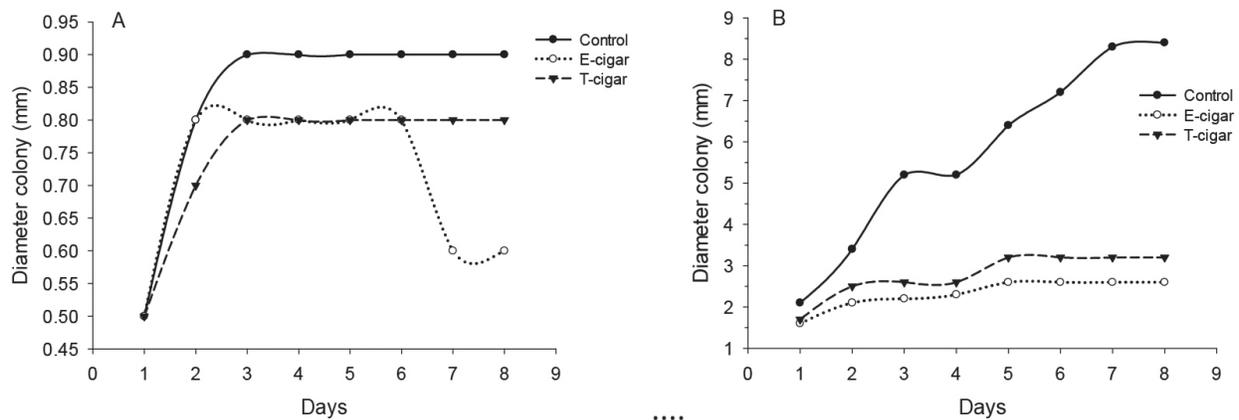


Fig. 1. Growth of *S. pneumoniae* (A) and *K. pneumoniae* (B) on Mac Conkey Agar at 37 °C with and without exposure of cigar smoke. The *K. pneumoniae* and *S. pneumoniae* were grown on Mac Conkey agar at 37 °C. Each type of culture was treated with two types of treatment (exposed by the smoke of e-cigar and t-cigar), while one was the control (no expose).

incubation). The growth pattern of the *S. pneumoniae* and *K. pneumoniae* with exposure of e-cigar and t-cigar smoke were shown in Figure 1.

The inhibition power of cigarette smoke on pathogenic bacteria

Using two pathogenic bacteria (*S. pneumoniae* and *K. pneumoniae*) in vitro, the smoke of e-cigar showed better inhibition than t-cigar smoke (Table 1).

This result showed oppositely to the previous report (Bagaitkar *et al.*, 2008; Shen *et al.*, 2016; Cogo *et al.*, 2008), which showed that conventional cigarette smoke could facilitate the growth of *Pneumonia* bacteria. The exposed of e-cigar and t-cigar smoke inhibited the growth of *S. pneumoniae* and *K. pneumoniae*, but e-cigar smoke showed more significant inhibition than t-cigar. Vainio-Kaila *et al.*

(2017) demonstrated that the e-cigar smoke inhibited the *E. coli*, *S. pneumoniae* and slightly on *S. enterica* serovar Typhimurium. The higher inhibition power (IP) of the e-cigar smoke in this result on the growth of *S. pneumoniae* and *K. pneumoniae* may be caused by various substances found in the e-cigar smoke like propylene glycol, candium and chromium oxide. The propylene glycol showed bactericidal activity against *S. mutans*, *E. faecalis* and *E. coli* of 50, 25 and 50%, respectively (Nalawade *et al.*, 2015). Candium of 100 µg mL⁻¹ showed inhibition against *K. pneumoniae* of 46, 68, 83 and 93% following the incubation at 24, 48, 72 and 96 hours, respectively (Shamim and Rehman, 2012). The inhibition against *K. pneumoniae* was also shown by chromium oxide (Cr₂O₃) nanoparticles (Sangwan and Kumar (2017).

Table 1. Inhibition Power (IP, %) of electric (e-cigar) and tobacco cigarette (t-cigar) smoke on *S. pneumoniae* and *K. pneumoniae*

Incubation day of	IP of <i>S.pneumoniae</i>		IP of <i>K. Pneumoniae</i>	
	e-cigar	t-cigar	e-cigar	t-cigar
1	0.00	0.00	23.81	19.05
2	0.00	12.50	38.24	26.47
3	11.11	11.11	57.69	50.00
4	11.11	11.11	55.77	50.00
5	11.11	11.11	59.38	50.00
6	11.11	11.11	63.89	55.56
7	33.33	11.11	68.67	61.45
8	33.33	11.11	69.05	61.90

Note: E-cigar liquid composted of grade vegetable glycerin, usp propylene glycol, organic flavouring, water and nicotine. T-cigar composted of 31 mg of tar, 2.2 mg of nicotine per 1 gram Control was the media without smoke exposure. The bacteria were grown on Mac Conkey Agar media and Lowenstein Jensen Agar.

This study indicated that e-cigar smoke has the effect of suppressing the growth of *S. pneumoniae* and *K. pneumoniae*. However, this does not mean that e-cigar is safer to be consumed. The various chemicals contained in e-cigar smoke can adversely affect various human body systems. The propylene glycol and glycerol can accumulate and cause lactic acidosis, CNS depression, coma, hypoglycemia, seizures, and haemolysis (Lim *et al.*, 2014). Short exposure to propylene glycol mist from artificial smoke generators may cause acute ocular and upper airway irritation in non-asthmatic subjects and a few may also react with cough and slight airway obstruction (Wieslander *et al.*, 2001).

The Committee on the Review of the Health Effects of Electronic Nicotine Delivery Systems of the US National Academy of Sciences summarised that humectants in e-cigar were causing a dry mouth and throat. The aerosol from the e-cigar contains glycerol, aldehyde, flavouring agent and nitrosamines which could cause an impact on squamous metaplasia of the epiglottis, suppressed spermatogenesis, irritation to the respiratory tract, irritant and toxicity. The tobacco-specific nitrosamines (TSNAs) is a potent carcinogenic chemical, which its exposure can cause serious health effects like neurotoxicity, cardiovascular disease, lung cancer, allergic dermatitis, increased mitochondrial oxidative stress and DNA fragmentation (Eaton *et al.*, 2018).

CONCLUSION

The exposure of e- and t-cigar smoke inhibited the growth of *S. pneumoniae* and *K. pneumoniae* colonies. The e-cigar smoke showed higher inhibition against both bacteria types. However, the two types of smoke showed a higher inhibition effect against *K. pneumoniae* growth. Even the e-cigar showed good inhibition effect against the two bacteria types, due to the adverse reports of the chemicals in humectant of the e-cigar on the human health, this study did not state that e-cigar is safer than t-cigar.

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