Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22 (2) : 2020 : 243-247

ISSN-0972-3005

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

IWAN MUHAMAD RAMDAN[1], KRISHNA PURNAWAN CANDRA[2] AND YADI YASIR[3]

[1]Faculty of Public Health, Mulawarman University, Samarinda, 75119, Indonesia,[2]Department of Agricultural Products Technology, Faculty of Agriculture, Mulawarman University, Samarinda, 75119, Indonesia.
[3]Department of Microbiology, Faculty Medicine, Mulawarman University, Samarinda, 75119, Indonesia

(Received 16 November, 2019; accepted 31 December, 2019)

Key words : *Electric cigarette, Tobacco cigarette, S. pnemoniae, K. pnemoniae and M. tuberculosis.*

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. pnemonia*e colonies were smaller than *K. pnemoniae*. 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), nicotinergic 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

***Corresponding author's email**: iwanmuhamadramdan@gmail.com

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 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 nicotineper 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 (%) = ((Dc-Ds)/Dc)× 100, where Dc is the diameter of growth in control plate and Ds 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 ahose, 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 ^{o}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 cigarrettes 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

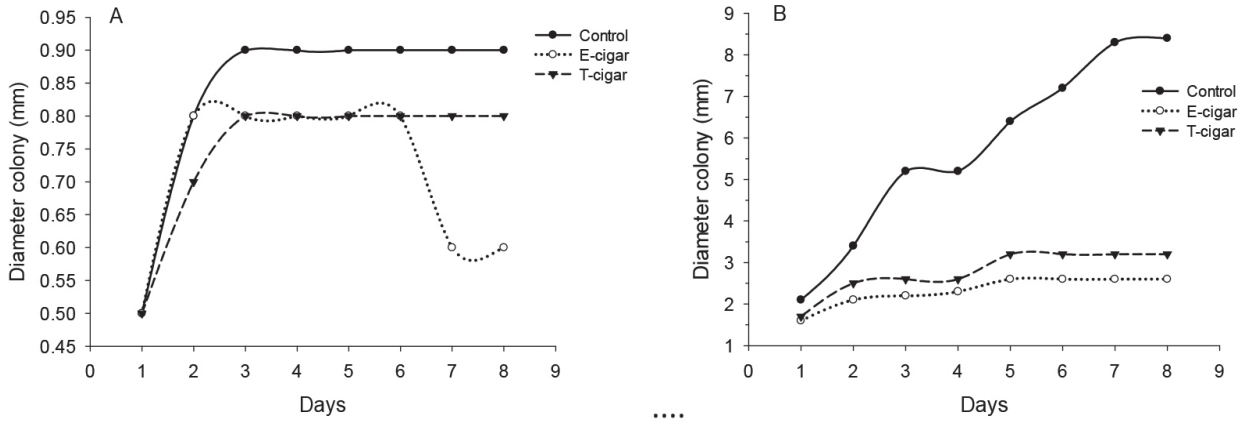

Fig. 1. Growth of *S. pneumoniae* (A) and *K. pneumoniae* (B) on Mac Conkey Agar at 37 °C with and without exposure of cigar smoke. The *K. pneumoniae* and *S. pneumoniae* were grown on Mac Conkey agar at 37 °C. Each type of culture was treated with two types of treatment (exposed by the smoke of e-cigar 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 cigarrette 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 (Cr_2O_3) 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 *S.pneumoniae*		IP of *K. Pneumoniae*	
	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 gramControl 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 slightairway 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. pnemoniae* 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.

Grant information

This work is supported by Islamic Development Bank (IDB), Development of Four Higher Education Institution, Project implementation unit of Mulawarman University of Indonesia 2019.

ACKNOWLEDGEMENTS

The authors are grateful to the Rector of Mulawarman University, Director of PIU Mulawarman University-Islamic Development Bank, and Head and Laboratory Assistant of the Biological Molecular and Immunology Laboratory, Faculty of Medicine, Hasanudin University.

REFERENCES

Bagaitkar, J., Demuth, D. R. and Scott, D. A. 2008. Tobacco use increases susceptibility to bacterial infection. *Tobacco Induced Diseases*. 4(12): 1–10. https://doi.org/10.1186/1617-9625-4-12

Balouiri, M., Sadiki, M. and Ibnsouda, S. K. 2016. Methods for *In vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 6(2): 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Baskaran, V., Murray, R. L., Hunter, A., Lim, W. S. and McKeever, T. M. 2019. Effect of tobacco smoking on the risk of developing community acquired pneumonia: A systematic review and meta-analysis. *Plos One*. 14(7) : e0220204. https://doi.org/10.1371/journal.pone.0220204

Bauld, L., Mackintosh, A. M., Eastwood, B., Ford, A., Moore, G., Dockrell, M. and McNeill, A. 2017. Young people's use of e-cigarettes across the united kingdom: Findings from five surveys 2015–2017. *International Journal of Environmental Research and Public Health*. 14(9). https://doi.org/10.3390/ijerph14090973

Bedi, R. S. 2006. Community Acquired Pnemonia - Typical or Atypical/? *Lung India. 23*(December 2004), 130–131.

Brozek, G., Jankowski, M., Shpakou, A., Poznanski, M., Klimackaya, L., Loginovic, J. and Jan, Z. 2018. The prevalence of e-cigarette and cigarette smoking among students in Central and Eastern Europe - preliminary results of YUPESS study. *European Respiratory Journal*. 52(Supl. 62). https://doi.org/0.1183/13993003.congress-2018.PA4543

Chai, Q., Zhang, Y. and Liu, C. H. 2018. Mycobacterium tuberculosis: An adaptable pathogen associated with multiple human diseases. *Frontiers in Cellular and Infection Microbiology*. 8(MAY), 1–15. https://doi.org/10.3389/fcimb.2018.00158

Cogo, K., Franz Montan, M., Bergamaschi, C. D. C., Andrade, E. D., Rosalen, P. L. and Groppo, F. C. 2008. *In vitro* evaluation of the effect of nicotine, cotinine, and caffeine on oral microorganisms. *Canadian Journal of Microbiology*. 54(6): 501–508. https://doi.org/10.1139/W08-032

Cuadra, G. A., Smith, M. T., Nelson, J. M., Loh, E. K. and Palazzolo, D. L. 2019. A Comparison of Flavorless Electronic Cigarette-Generated Aerosol and Conventional Cigarette Smoke on the Survival and Growth of Common Oral Commensal Streptococci. *International Journal of Environmental Research and Public Health*. 16(1669) : 1–14.

Eaton, D. L., Alberg, A. J., Goniewecz, M., Leventhal, A., Manautou, J. E., McGrath-Morrow, S. and Miech, R. 2018. *Public Health Consequences of E-Cigarettes*. (K. Stratton, L. Y. Kwan, & D. L. Eaton, Eds.). Washington DC: The National Academies Press. https://doi.org/10.17226/24952

Flouris, A. D., Chorti, M. S., Poulianiti, K. P., Jamurtas, A. Z., Kostikas, K., Tzatzarakis, M. N. and Koutedakis, Y. 2013. Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. *Inhalation Toxicology*. 25(2) : 91–101. https://doi.org/10.3109/08958378.2012.758197

Goniewicz, M. L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J. and Benowitz, N. 2013. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tab Control*. 1: 3–9. https://doi.org/10.1136/tobaccocontrol-2012-050859

Grana, R., Benowitz, N. and Glantz, S. A. 2014. E-cigarettes: A scientific review. *Circulation*. 129(19) : 1972–1986. https://doi.org/10.1161/CIRCULATIONAHA.114.007667

Indonesian Ministry of Health. 2018. *Tuberkulosis*. *Infodatin (pusat data dan informasi kesehatan RI)* (Vol. 2). https://doi.org/2442-7659

Higham, A., Bostock, D., Dungwa, J. V. and Singh, D. 2018. The effect of electronic cigarette and tobacco smoke exposure on COPD bronchial epithelial cell inflammatory responses. *Internatioanal Journal of COPD*. 13 : 989–1000.

Jee, S. H., Golub, J. E., Jo, J., Park, I. S., Ohrr, H. and Samet, J. M. 2009. Smoking and risk of tuberculosis incidence, mortality, and recurrence in South Korean men and women. *American Journal of Epidemiology*. 170(12) : 1478–1485. https://doi.org/10.1093/aje/kwp308

Lim, T. Y., Poole, R. L. and Pageler, N. M. 2014. Propylene glycol toxicity in children. *The Journal of Pediatric Pharmacology and Therapeutics*. 19(4) : 277–27782. https://doi.org/10.5863/1551-6776-19.4.277

McMillen, R. C., Jd, M. A. G., Shaefer, R. M. W., Jonathan, P. and Klein, J. D. 2015. Original investigation Trends in Electronic Cigarette Use Among U. S. Adults/: Use is Increasing in Both Smokers and Nonsmokers. *Nicotine & Tobacco Research*. 17(10) : 1195–1202. https://doi.org/10.1093/ntr/ntu213

Nalawade, T., Sogi, S. P. and Bhat, K. 2015. Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms. *Journal of International Society of Preventive and Community Dentistry*. 5(2): 114. https://doi.org/10.4103/2231-0762.155736

Palipudi, K. M., Mbulo, L., Morton, J., Bunnell, R., Blutcher-nelson, G., Kosen, S. and Al, K. A. 2016. Awareness and Current Use of Electronic Cigarettes in Indonesia, Malaysia, Qatar, and Greece: Findings From 2011– 2013 Global Adult Tobacco Surveys. *Nicotine*. 18(4) : 501–507. https://doi.org/10.1093/ntr/ntv081.Awareness

Qasim, H., Karim, Z. A., Rivera, J. O., Khasawneh, F. T. and Alshbool, F. Z. 2017. Impact of Electronic Cigarettes on the Cardiovascular System. https://doi.org/10.1161/JAHA.117.006353

Ramezani, M., Aemmi, S. Z. and Moghadam, Z. E. 2015. Factors Affecting the Rate of Pediatric Pneumonia in Developing Countries/: a Review and Literature Study. *Int Journal Pediatrics*. 3(24) : 1173–1181.

Rudan, I., Boschi-pinto, C., Biloglav, Z. and Campbell, H. 2008. Epidemiology and etiology of childhood pneumonia. *World Health Organization*. 86(5). https://doi.org/10.2471/BLT.07.048769

Sangwan, P. and Kumar, H. 2017. Synthesis, characterization, and antibacterial activities of chromium oxide nanoparticles against *Klebsiella pneumoniae*. *Asian Journal of Pharmaceutical and Clinical Research*. 10(2) : 206–209. https://doi.org/10.22159/ajpcr.2017.v10i2.15189

Shamim, S. and Rehman, A. 2012. Cadmium resistance and accumulation potential of *Klebsiella pneumoniae* strain CBL-1 isolated from industrial wastewater. *Pakistan Journal of Zoology*. 44(1) : 203–208.

Shen, P., Morissette, M. C., Vanderstocken, G., Gao, Y., Hassan, M., Roos, A. and Stämpfli, M. R. 2016. Cigarette smoke attenuates the nasal host response to *Streptococcus pneumoniae* and predisposes to invasive pneumococcal disease in mice. *Infection and Immunity*. 84(5) : 1536–1547. https://doi.org/10.1128/IAI.01504-15

Stewart, C. J., Auchtung, T. A., Ajami, N. J., Velasquez, K., Smith, D. P., La, R. De, and Petrosino, J.F. 2018. Effects of tobacco smoke and electronic cigarette vapor exposure on the oral and gut microbiota in humans: a pilot study. *Peer J*. 1–16. https://doi.org/10.7717/peerj.4693

Vainio-Kaila, T., Hänninen, T., Kyyhkynen, A., Ohlmeyer, M., Siitonen, A. and Rautkari, L. 2017. Effect of volatile organic compounds from *Pinus sylvestris* and *Picea abies* on *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Salmonella enterica* serovar *Typhimurium*. *Holzforschung*. 71(11) : 905–912. https://doi.org/10.1515/hf-2017-0007

Wieslander, G., Norbäck, D. and Lindgren, T. 2001. Experimental exposure to propylene glycol mist in aviation emergency training: Acute ocular and respiratory effects. *Occupational and Environmental Medicine*. 58(10) : 649–655. https://doi.org/10.1136/oem.58.10.649.