Short Communication

Bactericidal Effects of Povidone-iodine and Chlorhexidine Gluconate on Coagulase-positive Staphylococci

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Abstract

We aimed to evaluate the *in vitro* bactericidal efficacy of two routine antiseptics used in veterinary hospital, povidone-iodine and chlorhexidine gluconate in isopropanol (CGI),against canine coagulase-positive staphylococci (CoPS). Twenty CoPS were divided into 5 methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and 5 isolates each of *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. *coagulans* defined as methicillin-susceptible CoPS (MSCoPS). The bactericidal efficacy was determined by broth microdilution according to European Standard EN 1656:2000 at concentrations of 0.1, 1 and 10% PI and 0.5, 1 and 2% CGI for 15s, 30s, 45s, 1 min, 3 min and 5 min, respectively. There was no difference in susceptibility values between the strains. Regarding the fastest bactericidal effects, all tested CoPS were killed with 0.1% PI within 45s and 0.5% CGI within only 15s.

Keywords: povidone-iodine, coagulase-positive staphylococci, chlorhexidine gluconate in isopropanol, methicillin-resistant, methicillin-susceptible

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Introduction

Coagulase-positive staphylococci (CoPS) is gram positive cocci bacteria comprising the three members of CoPS on dog skin; *Staphylococcus (S.) pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. *coagulans*. Methicillin-resistant coagulase-positive staphylococci (MRCoPS) poses prolonged infection in canine allergic dermatitis that may be a source of spreader in hospital and animal household (Windahl et al., 2012). For public health concern, these bacteria have been reported as the cause of secondary infection in immunocompromised and operated human patients (Bergstrom et al., 2012). To reduce the impact of MRCoPS dissemination in veterinary hospital, use of antiseptic is the empirical tool for decontamination and wound management.

Povidone-iodine (PI) and chlorhexidine gluconate (CG) are common antiseptics in veterinary and human hospitals. The antimicrobial agent of PI is composed of three ion including iodine (I_2), hypoiodous acid (HOI) and iodide (I^-) (Rackur, 1985) that are produced from iodine reaction in aqueous solution. In veterinary practice, PI is used for pre-surgical skin preparation (Osuna et al., 1990) and treatment of infectious wound (Sanchez et al., 1988). However, the knowledge of exposing time and concentrations to kill *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. *coagulans* isolated from dog has not been revealed.

Chlorhexidine gluconate is a chemical compound against bacteria, fungi and enveloped virus. By bactericidal mechanism, the positively-charged ion of CG is attached tothe negatively-charged site of cell wall, leading to cell death (McDonnell and Russell, 1999). Practically, mixing with alcohol solution can increase the efficacy of bactericidal activity of CG (Sakuragi et al., 1995). However, the bactericidal effect of chlorhexidine gluconate in isopropanol (CGI) on common CoPS on dog skin has not been reported. Moreover, time of exposure and concentrations of each disinfectant compound are important parameters that cause difference in bactericidal effects on each microorganism. In this study, we aimed to evaluate the *in vitro* bactericidal efficacy of PI and CGI against canine CoPS member in different concentrations and exposing times.

Materials and Methods

Antiseptics: The antiseptic agents chosen for this study were routinely used at the Veterinary Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Betadine® solution (Reg. No. 1A 675/31, IDS manufacturing LTD., Pathumthani, Thailand) contains 10% of PI (Mundidone®, Netherlands) in aqueous solution. Betadine was diluted to 1 and 0.1% by ultrapure water. All solutions (10, 1 and 0.1%) were freshly prepared before determination.

Q-Bac2A® (Reg. No. 1-1-03-02-13-00463, Pose Health Care Limited, Bangkok, Thailand) is composed of 2% CGI. CGI was prepared at 2, 1 and 0.5% dilution by ultrapure water.

Bacterial isolation and identification: A total of 20 isolates consisting of 5 methicillin-resistant *S. pseudintermedius* (MRSP) and 15 MSCoPS; 5 samples each of *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp. *coagulans*; were derived from a previous study of Chanchaithong and Prapasarakul (2011). The number of population referred to the study of Sakuragi et al. (1995). All bacteria were identified by an approved biochemical analysis (Chanchaithong and Prapasarakul, 2011) and confirmed by multiplex PCR (Sasaki et al., 2010). The methicillin-resistant trait was characterized by oxacillin disk diffusion test (CLSI, 2013) and *mecA* identification (Strommenger et al., 2003), whereas the susceptible strains were defined by these negative results. The bacteria were prepared by cultivation on tryptic soy agar (TSA) containing 5% sheep blood at 37°C overnight.

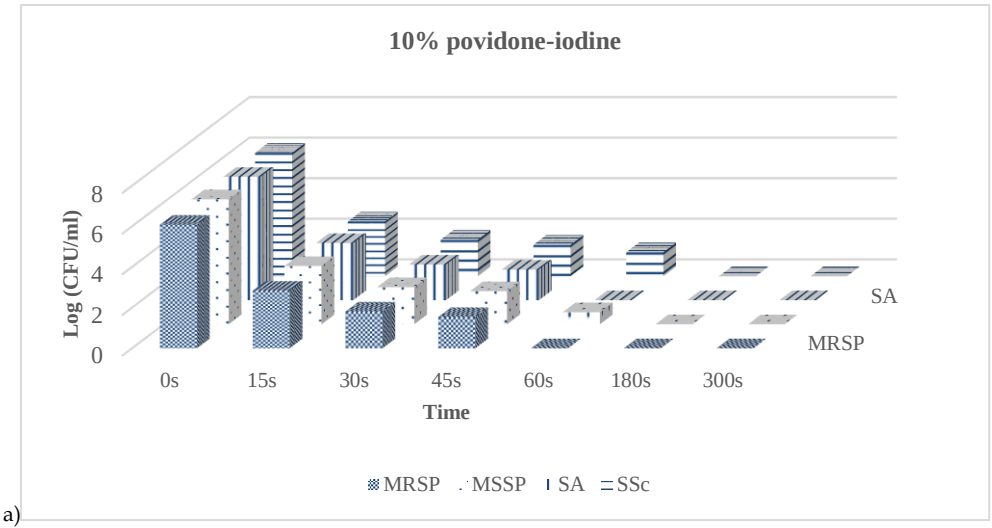
Bactericidal determination: Broth microdilution was modified from the European Standard EN 1656:2000 (2000) and Banovic et al. (2013). Briefly, the bacterial concentration was adjusted at 0.5 McFarland using a densitometer (DEN-1B McFarland Densitometer, Grant-bio®, Cambridgeshire, UK). The stock solutions of bacteria were finally adjusted to approximately 10^6 CFU/ml and the population was confirmed following ISO standard enumeration (ISO6888-1, 1999). One hundred microliters of bacterial suspension were mixed with 100 µl of chemical agent dilution in a 96-well sterile plate (Nunclon™ Delta Surface, Thermo Scientific, Jiangsu, China)(Banovic et al., 2013). After exposure at 15s, 30s 45s, 60s, 180s and 300s, reactions between the antiseptic and bacteria were stopped by mixing with 20 µl of the suspension with 180 µl of D/E neutralizer (BBL®, French) for 5 min. Thereafter,10 µl of the suspension were placed on 5% sheep blood and incubated at 37°C for 24 h (BSEN, 2000). *S. aureus* ATCC 6538 was used as the strain control. The procedure was repeated at least two times.

Statistical analysis: All statistical analyses were calculated by IBM SPSS Statistics Desktop version 22.0 (SPSS Inc.; Chicago, IL, USA). Difference in time-kill between MRSP and MSCoPS was analyzed by Chi-square statistic. Minimum bactericidal concentration (MBC) was described as the lowest concentration and minimal time that could kill all bacteria (BSEN, 2000).

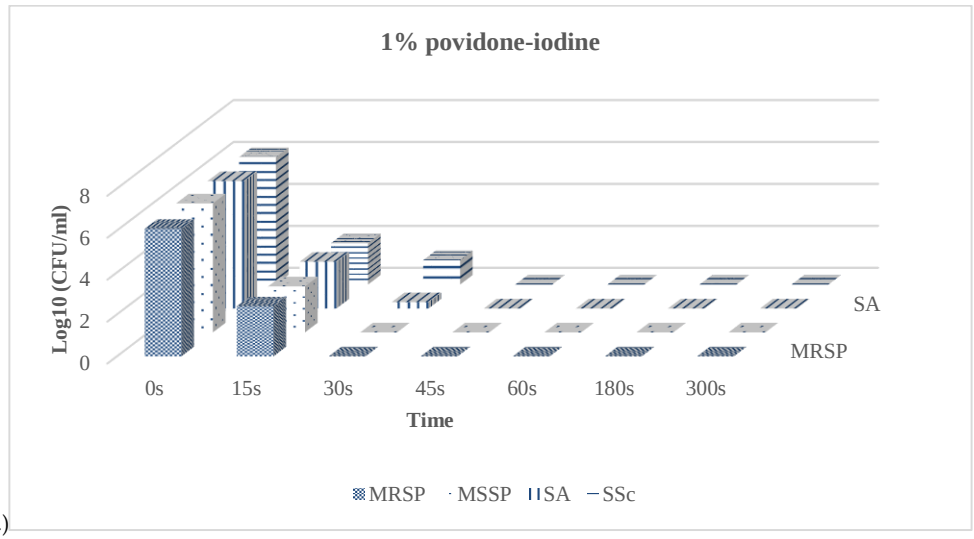
Results and Discussion

TheD/E neutralizer and ultrapure water did not affect bacterial growth. The bactericidal efficacy of PI against canine staphylococci is presented in Figure 1. At 10% PI, *S. schleiferi subsp. coagulans* and *S. pseudintermedius* were terminated within 180s while *S. aureus* and MRSP were killed within 60s. In veterinary practice, 10% PI is recommended as the most stable form of PI preparation due to its slow releasing property of polyvinylpyrrolidone (PVP) for at least 18 hours (Kunkle et al., 2014). At *0.1* and *1%* concentrations, PI eliminated both MRCoPS and MSCoPS within 45s. The results could be explained by the dilution phenomenon (Rackur, 1985). The dilution phenomenon is known as low in concentration but high in bactericidal activity (Berkelman et al., 1982).For

PI, 0.01-1% concentration could release the highest concentration of iodine, the main antimicrobial substrate (Rackur, 1985), and immediately react to bacterial cell membrane (Gottardi, 1985). However, this aqueous dilution was unstable (Mueller et al., 2012); it could not prolong bactericidal activity on applied site.In addition, the use of 0.1 and 1% PI was recommended for treatment of mouthwash (Higashitsutsumi et al., 1993), eye infection (Pinto et al., 2015) and pre-surgery preparation (Ferguson et al., 2003) in human and wound dressing in dogs (Sanchez et al., 1988). For all tested concentrations of PI, 3-minute exposure time was recommended for aseptic preparation such as on pre-surgical site (Reichman and Greenberg, 2009). A notable side effect of PI is the inhibition of human skin fibroblast growth (Balin and Pratt, 2002) and skin irritation in dogs (Osuna et al., 1990). However, reports on 0.1 to 10% PI side effect were very low in animal (Mueller et al., 2012).



a)



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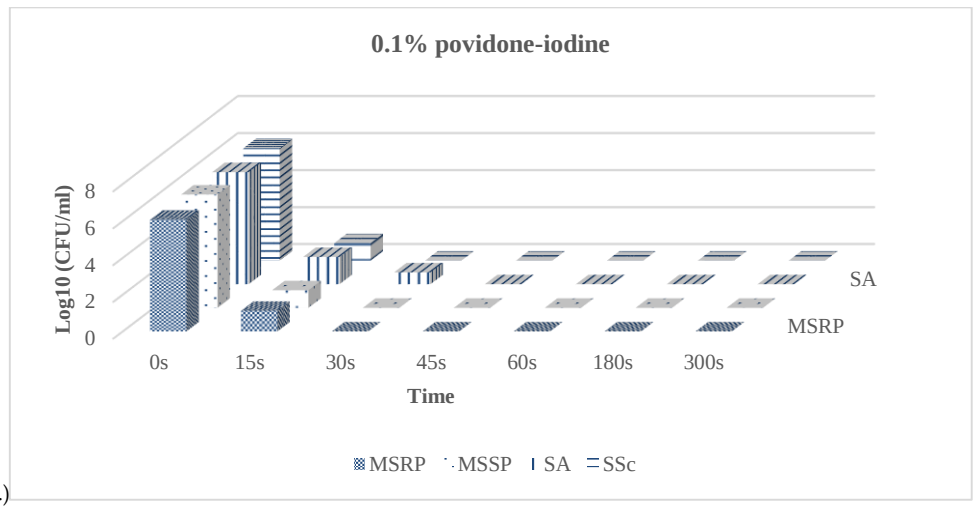

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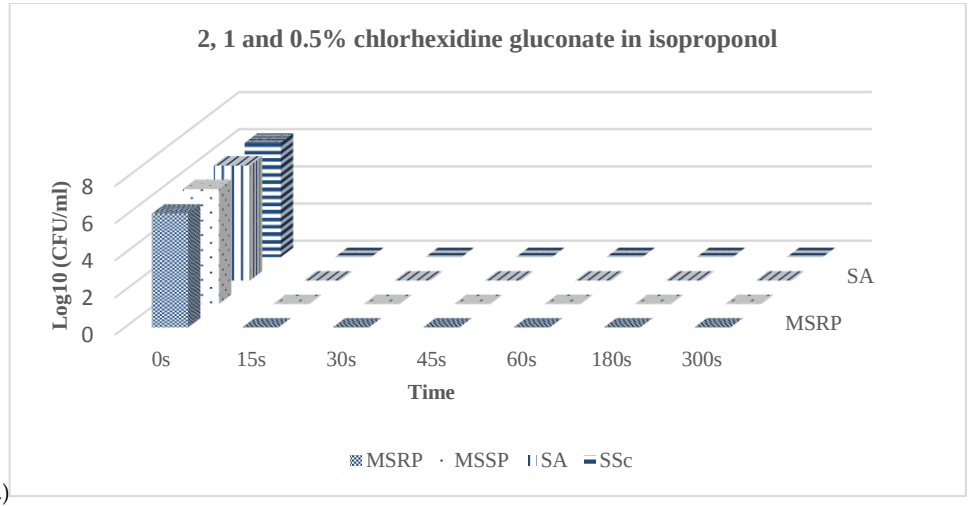

d.)

Figure 1 Exposing time and concentration of PI to methicillin-resistant *S. pseudintermedius* (MRSP), methicillin-susceptible *S. pseudintermedius* (MSSP), *S. schleiferi* subsp. *coagulans* (SSc) and *S. aureus* (SA); a) 10% *povidone-iodine*, b) 1% povidone-iodine,c) 0.1% povidone-iodine and d.) 0.5-2% chlorhexidinegluconate in isopropanol.

In this study,the concentration of 0.5-2.0% CGI could kill all MRSP and MSCoPS within 15s.There was no difference between the methicillin-susceptible and methicillin-resistant traits. In a previous study, CG without alcohol eliminated staphylococci over 5 min (Banovic et al., 2013). Due to the mixing with alcohol, this productcould eliminate much faster. In veterinary practices, 0.5% CG is the active ingredient in antiseptic shampoo in dogs (Kwochka and Kowalski, 1991)and is also easy to prepare for hygienic use in household and hospitals (Mueller et al., 2012). In veterinary clinic, the amount of 2% chlorhexidine could lead to an ototoxic effect in cats (Igarashi and Oka, 1988). Moreover, an important side effect of CGI is the breakdown of red blood cell (Gabler et al., 1987). Therefore, CGI is not recommended for treatment of open wound with a lot of capillary injury. However, there have been no reports on the adverse effect of 0.5-1% chlorhexidine on animal.

In summary, according to the results of this study, we suggest that 10% povidone-iodine should be exposed to CoPS for at least 3 min, and that 1:10 and 1:100 dilutions from its original concentration can increase the efficacy by shortening the time-kill to 45s. Moreover, at least 0.5% of CGI is recommended for bactericidal activity within 15s.

Acknowledgements

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บทคัดย่อ

ประสิทธิภาพของในการฆ่าเชื้อของยาฆ่าเชื้อ โพวิโดน ไอโอดีนและคลอเฮกซิดีน กลูโคเนตต่อเชื้อในกลุ่มแอกกูเลส โพสสิทีฟ สตาฟฟิลโลคอคไค

พรรณพิชญา ฟุ้งวิทยา และ ณุวีร์ ประภัสระกูล*

การทดลองนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพการฆ่าเชื้อของ povidone-iodine และ chlorhexidine gluconate ใน isopropanol (CGI) ในห้องทดลองต่อเชื้อ coagulase-positive staphylococci (CoPS) ที่เก็บได้จากสุนัข เชื้อจำนวน 20 ตัวอย่างแบ่งเป็นเชื้อดื้อยา methicillin-resistant *Staphylococcus pseudintermedius* 5 ตัวอย่างและ *S. pseudintermedius*, *S. aureus* และ *S. schleiferi* subsp. *coagulans* อย่างละ 5 ตัวอย่างที่พิสูจน์แล้วว่าเป็นเชื้อไม่ดื้อยา (methicillin-susceptibleCoPS, MSCoPS) ในการทดสอบประสิทธิภาพของสารทั้งสองชนิดใช้วิธี broth microdilution ร่วมกับ European Standard EN 1656:2000 ที่ความเข้มข้นที่ 0.1, 1 และ 10% PI และ 0.5, 1 และ 2% CGI ที่เวลา 15 วินาที, 30 วินาที, 45 วินาที, 1 นาที, 3 นาที และ 5 นาที ตามลำดับการทดลองไม่พบความแตกต่างของเวลาที่ใช้ในการฆ่าเชื้อแต่ละสายพันธุ์ ทั้งนี้ประสิทธิภาพการฆ่าเชื้อ CoPS ที่เร็วที่สุด คือ ที่ 0.1% PI ภายใน 45 วินาที และ 0.5% CGI ภายใน 15 วินาทีผลการทดลองนี้สามารถนำไปเป็นแนวทางในการใช้สารฆ่าเชื้อทั้งสองต่อไป

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