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Bactericidal efficacy of potassium peroxymonosulfate under various concentrations, organic material conditions, exposure timing and its application on various surface carriers

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ABSTRACT. Four concentrations of potassium peroxymonosulfate (PPMS) were evaluated for bactericidal activities and indicated that the concentration is less than the manufacturing-recommended concentrations, must extend the exposure time for bacterial inactivation. However, even with and without of organic material contamination, did not show marked inactivation difference. In addition, all concentrations were inactivated on all carrier surfaces within 30 sec, except on rubber where inactivation occurred within 1 min. However, quaternary ammonium compounds were inactivated on stainless steel and plastic within 1 min and 30 sec, respectively, but not inactivated within 5 min on rubber surfaces. Conclusion, PPMS inactivated bacteria under optimal concentration, organic material conditions, exposure timing and on carrier surfaces which can useful as an alternative disinfectant for biosecurity enhancement.

KEY WORDS: bactericidal, biosecurity, carrier surface, disinfectant, potassium peroxymonosulfate

Normally, animal food products especially eggs, meat and milk, have been implicated as vehicles of one or more pathogens causing food-borne illness, [1, 4] such as salmonellosis and colibacillosis [10]. Generally, bacterial transmission in poultry occurs from flock to flock via the transportation of cages containing faeces from previously-transported birds, especially those with dirty floor surfaces [11]. Totton *et al*. [29] reported the routes of salmonella enteritis spreading by direct contact, such as infected animals and fomites, including plastic poultry transport cages [17, 18]. In addition, several pathogens can spread through footwear worn by farmers, employees and farm visitors [13, 17]. Therefore, biosecurity and disinfectant applications are most important for disease prevention and control, especially in the poultry industry [24].

However, several factors pertaining to poultry farms, such as organic material contamination, various carrier surfaces and exposure timing between disinfectant and pathogens, are responsible for altering the kinetics of disinfection [6, 16]. Several researchers, such as Bloomfield *et al*. [2] and Payne *et al.* [15], have evaluated the bactericidal efficacy using laboratory bacterial suspension, especially disinfectant testing.

There have been several trials to explain alternative materials for biosecurity enhancement, which do not interfere, even with contaminated organic materials. Several researchers have shown alkaline agents can inactivate bacteria, even in the presence of organic materials, such as bioceramic powder [26], slaked lime, scallop shell powder [28], calcinated egg shell [14], calcium hydroxide [20] and fresh charcoal ash [19]. However, several acidic agents, such as potassium monopersulfate, can inactivate both bacteria and viruses, even in the presence of organic material [22, 23].

The aim of the present study is to evaluate the efficacy of potassium peroxymonosulfate (PPMS) against rifampicin-resistant *Salmonella* Infantis (rif-SI), *Escherichia coli* (*E. coli*) and *Salmonella* Infantis (SI) under various concentrations, organic material

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conditions and exposure timing, including efficacy of bacterial inactivation on various surface carriers, such as stainless steel, rubber and plastic.

The PPMS powder (54.45% of PPMS; BIOX®, Biogénesis Bagó, Buenos Aires, Argentina; batch number 009C; expiation date 02/2020), was freshly prepared at manufacturer-recommended concentrations as 1% (weight/volume), namely 1X, 0.5% (0.5X), 0.25% (0.25) and 0.125% (0.125X) for bactericidal testing. In order to represent organic material conditions at 5%, 500 *µl* of fetal bovine serum (FBS) was added to 10 m*l* of each concentration before testing.

In the present study, rif-SI, *E. coli* and SI were sub-cultured onto deoxycholate hydrogen sulphide lactose (DHL) agar and incubated into 37°C incubator for overnight. The bacterial colony was picked up and cultivated in Luria-Bertani (LB) medium (1% Bacto Tryptone, 0.5% Bacto Yeast Extract and 1%NaCl, pH 7.4) [13]. Organic materials were removed from the culturing bacteria organic materials by washing and centrifugation for 2 times, then re-suspended with phosphate buffer saline (PBS) before testing.

The mixture between 1 M Tris hydrochloride (Tris-HCl) at pH 7.4 and FBS in the ratio 7:3, namely blocking solution, was used for PPMS neutraization or stop activities of bacterial inactivation.

Four hundred microliters of each PPMS concentration, were mixed with 100 *µl* of each bacterium, then incubated at room temperature for the indicated time, namely 5 sec and 30 sec, 1 min, 3 min, 5 min, 10 min or 15 min. After the incubation period, the solution mixture was immediately neutralized by 500 *µl* of the blocking solution, then titrated onto DHL agar plates for bacterial titration. All inoculated petri-disks were incubated at 37°C incubator for overnight, and the number of colonies was counted after 24-hr post inoculation. The bacteria titer was calculated in the colony-forming units (CFU)/m*l*. To conform the neutralizing efficacy of the blocking solution, it was added to each solution sample before adding bacteria, namely at 0 sec. Each treatment in the present study was tested in triplicate, and the titers were shown in mean with standard error (SE).

Three different surface carriers such as stainless steel, rubber and plastic, were purchased from local markets and used for the testing of bactericidal efficacy on contaminated carriers. All the surface carrier sheets were prepared into small pieces of 5.0 × 5.0 cm. Firstly, the pieces were washed with tap water to remove visible dirt from the carrier surfaces. Then, all carriers were sterilized by autoclave at 121°C for 15 min, dried and stored into a 60°C incubator until testing.

During testing, each sterile carrier was placed separately in a 90 mm petri-dish under a level 2 biological safety cabinet, then 100 *µl* of rif-SI containing 5%FBS, was inoculated onto the surfaces of each carrier. After that, the bacterial suspension was spread and kept inside the biologically safety cabinet for 5 min. These artificially contaminated carriers were tested the bacterial inactivation using 4 concentrations of PPMS, and compared with the fourth generation of quaternary ammonium compounds (QAC) as manufacturer-recommended concentration.

Following that, 500 *µl* of each sample concentration was added onto each type of contaminated surface and incubated for 30 sec, and 1 min, 3 min and 5 min. After incubation determination, each tested sample was neutralized or stop bacterial inactivity by placing the carrier into a stomacher bag containing 2 m*l* of the blocking solution. Subsequently, each carrier surface was vigorously rubbed by hand over the bag, and scraped with a sterile pipette tip to remove the bacteria from the carrier surfaces into the blocking solution. Then, the resulting solution was transferred separately from the stomach bag into a microtube and diluted in 10-fold serial dilution using PBS and cultured onto DHL agar plates for bacterial titration. Together, as a control or non-treatment, 500 *µl* of double distilled water (dW_2 control) was added onto each contaminated carrier and kept for 3 min then placed to blocking solution into stomacher bag, bacterial removing and titration, respectively.

The reduction factor (RF) was used to determine the bacteria inactivation. The RF was calculated using the following equation: $RF=t_{pc} - t_a$ −where: t_{pc}=the titer converted into an index in $\log_{10}$ of the bacteria control or dW_2 sample for aqueous phase and on carrier, respectively, and t_a=the converted titer, an index in $\log_{10}$ of the recovered bacteria from the treated sample. Bacteria inactivation was considered effective when RF was greater than or equal to 3 (3 $\log_{10}$) [9, 25, 27].

In the present study, the RF was analysed independently and shown as mean ± standard error (SE). However, the statistically significant determination was classified as different when the *P* value was less than 0.05, using the one-way analysis of variance (ANOVA) *post hoc* test (SPSS, Armonk, NY, USA) between the positive control or dW_2 control and treatment group.

As shown in Table 1, PPMS could inactivate rif-SI, *E. coli* and SI in the aqueous phase, even in the absence or presence of organic materials. The concentration at 1X, 0.5X, 0.25X and 0.125X could inactivate rif-SI in the absence of organic materials within 5 sec, 30 sec, 30 sec and 30 sec, respectively. However, in the presence of organic material, inactivation occurred within 5 sec, 30 sec, 30 sec, and 10 min, respectively. The *E. coli* inactivation without organic material contamination, could be effected within 5 sec, 5 sec, 30 sec and 30 sec, respectively. However, in the presence of organic material, inactivation took place within 5 sec, 30 sec, 30 sec and 1 min, respectively (Table 1). The SI was inactivated by PPMS in the absence of organic materials within 5 sec, 30 sec, 30 sec and 30 sec respectively. However, even in the presence of organic material, SI could be inactivated within 5 sec, 5 sec, 30 sec, and 5 min, respectively (Table 1).

As shown in Table 2, the recovered amount of bacteria from rif-SI contaminated carrier was presented in the range of dW_2 control as 7.61 to 8.14 $\log_{10}$ CFU/m*l* from all carriers. All concentrations of PPMS could inactivate rif-SI on all carriers within 30 sec, except on rubber surfaces, where inactivation took place within 1 min. However, QAC could inactivate on stainless steel and plastic surfaces within 1 min and 30 sec, respectively, while on rubber surfaces, inactivation could not occur within 5 min.

This BIOX® is contains the active ingredient as PPMS at 54.45%, and synonymous is potassium monopersulfate (PMPS). PPMS belongs to the disinfectant group of oxidizing agents and its oxidising mechanism includes the destruction of proteins, especially disrupting structural protein of bacteria. Shukur and Adnan [22] reported that PPMS in Oxone® and Dupont™ breaks the chorine-ammonia bond during chlorine combined with ammonia, without the addition of chlorine in the swimming pool, and treated water for cleaning water [22]. These data indicated that PPMS is safe for human. Bolder [3] was descripted that the 21.41%PPMS,

Table 1. Log_{10} colony-forming units (CFU)/m*l* (mean ± SE) of rifampicin-resistant *Salmonella* Infantis (rif-SI), *Escherichia coli* (*E. coli*) *and Salmonella* Infantis (SI) subsequent to treated by potassium peroxymonosulfate at manufacturing-recommended concentration (1X), 0.5X, 0.25X or 0.125X even in the absence or presence of organic materials

		1X		0.5X		0.25X		0.125	
		Absence[a)]	Presence[b)]	Absence	Presence	Absence	Presence	Absence	Presence
rif-SI	t_{pc}[c)]	8.84 ± 0.06	8.84 ± 0.06	8.84 ± 0.06	8.84 ± 0.06	8.77 ± 0.29	8.93 ± 0.25	8.77 ± 0.29	8.82 ± 0.34
	0 sec[d)]	8.81 ± 0.04	8.81 ± 0.15	8.69 ± 0.07	8.84 ± 0.09	8.91 ± 0.20	9.05 ± 0.23	8.95 ± 0.13	8.57 ± 0.86
	5 sec[e)]	4.25 ± 1.85*	4.96 ± 2.09*	6.31 ± 0.72	6.72 ± 1.46	6.80 ± 0.36	7.72 ± 0.21	7.78 ± 0.31	NT
	30 sec	<2.60 ± 0.00**	<2.60 ± 0.00**	2.70 ± 0.17*	2.80 ± 0.35*	<2.6 ± 0.00**	5.88 ± 1.28*	2.92 ± 0.55*	NT
	1 min	NT	NT	NT	NT	NT	3.88 ± 2.21*	<2.6 ± 0.00**	7.60 ± 0.00
	5 min	NT	NT	NT	NT	NT	NT	NT	6.72 ± 1.07
	10 min	NT	NT	NT	NT	NT	NT	NT	4.48 ± 1.11*
	15 min	NT	NT	NT	NT	NT	NT	NT	3.04 ± 0.75*
E. coli	t_{pc}	8.04 ± 0.19	8.04 ± 0.19	8.15 ± 0.12	8.15 ± 0.12	7.89 ± 0.21	7.89 ± 0.21	7.89 ± 0.21	7.89 ± 0.21
	0 sec	7.81 ± 0.18	7.50 ± 0.39	7.89 ± 0.22	7.87 ± 0.06	7.94 ± 0.04	7.99 ± 0.10	8.25 ± 0.56	7.59 ± 0.60
	5 sec	<2.60 ± 0.00**	<2.60 ± 0.00**	4.06 ± 0.24*	5.57 ± 1.39	5.33 ± 0.51	6.13 ± 0.32	5.50 ± 1.18	NT
	30 sec	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.06 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	6.01 ± 0.22
	1 min	NT	NT	NT	NT	NT	NT	NT	4.80 ± 0.66*
	5 min	NT	NT	NT	NT	NT	NT	NT	<2.60 ± 0.00**
SI	t_{pc}	8.43 ± 0.07	8.43 ± 0.07	8.43 ± 0.07	8.43 ± 0.07	8.21 ± 0.06	8.10 ± 0.22	8.09 ± 0.23	8.04 ± 0.32
	0 sec	7.76 ± 0.05	8.32 ± 0.29	8.56 ± 0.13	8.47 ± 0.04	8.25 ± 0.10	8.10 ± 0.25	8.13 ± 0.26	7.94 ± 0.28
	5 sec	3.31 ± 1.22*	3.62 ± 0.92*	6.79 ± 0.33	3.62 ± 0.92*	6.75 ± 1.20	6.97 ± 0.89	7.37 ± 0.33	7.68 ± 0.11
	30 sec	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	3.40 ± 1.38*	4.46 ± 1.61*	3.78 ± 2.05*	6.90 ± 0.61
	1 min	NT	NT	NT	NT	NT	NT	NT	6.05 ± 0.15
	5 min	NT	NT	NT	NT	NT	NT	NT	<2.60 ± 0.00**

a) Absence of organic material. b) Presence of organic materials. c) The titer converted into an index in log_{10} of bacteria control. d) Blocking solution added before bacteria. e) The titer converted into an index in log_{10} of the recovered bacteria after indicated duration of treatment such as 5 sec, 30 sec, 1 min, 5 min 10 min and 15 min. NT: Not tested. *Inactivation regarded as effective when RF was greater than or equal to 3 and indicated to be statistical significant (P<0.05). **Bacterial titer <2.60 log_{10} CFU/m*l* indicates bacterial reduction to the undetectable level and significantly inactivating effective.

Table 2. Bactericidal efficacies of potassium peroxymonosulfate at manufacturing-recommended concentration (1X), 0.5X, 0.25X or 0.125X compared with quaternary ammonium compound (QAC) toward rifampicin-resistant *Salmonella* Infantis on various surface carriers

Disinfectant	Type of carrier	Concentration	dW_2 control (log_{10} CFU/m*l*)	Bacterial titer at different contact times (log_{10} CFU/m*l*)			
			3 min	30 sec	1 min	3 min	5 min
Biox	Stainless steel	1X[a)]	7.62 ± 0.38[e)]	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.5X[b)]	7.55 ± 0.01	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.25X[c)]	7.59 ± 0.00	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.125X[d)]	8.14 ± 0.95	4.52 ± 0.96*	3.77 ± 1.60*	2.90 ± 0.52*	<2.60 ± 0.00**
	Rubber	1X	7.66 ± 0.22	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.5X	7.62 ± 0.03	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.25X	7.61 ± 0.01	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.125X	7.72 ± 0.11	5.13 ± 0.43	3.77 ± 1.08*	2.77 ± 0.15*	<2.60 ± 0.00**
	Plastic	1X	7.65 ± 0.18	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.5X	7.49 ± 0.01	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.25X	7.69 ± 0.04	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**	<2.60 ± 0.00**
		0.125X	7.65 ± 0.10	4.60 ± 1.06*	2.81 ± 0.24*	<2.60 ± 0.00**	<2.60 ± 0.00**
QAC	Stainless steel	1X	7.75 ± 0.08	6.01 ± 1.05	3.01 ± 0.06*	3.14 ± 0.57*	4.24 ± 1.42*
	Rubber	1X	7.84 ± 0.14	6.84 ± 0.82	6.49 ± 0.48	5.84 ± 0.93	6.30 ± 1.15
	Plastic	1X	7.75 ± 0.11	2.79 ± 0.17*	3.30 ± 0.35*	2.80 ± 0.17*	2.70 ± 0.17*

a) Recommendation concentration as 1%. b) Half of recommendation concentration as 0.5%. c) 1/4 of recommendation concentration as 0.25%. d) 1/8 of recommendation concentration as 0.125%. e) The titer converted into an index in log_{10} of the recovered bacteria on surface carrier. *Single asterisk indicates effective bacterial reduction (≥3 log_{10} CFU/m*l*) **Bacterial titer ≤2.6 log_{10} CFU/m*l* indicates bacterial reduction to the undetectable level. Both effective and undetectable level bacterial reductions are significantly different (P<0.05) from dW_2 control titer.

namely Virkon-S®, has been used as a veterinary disinfectant and stringent EU legislation on Salmonella control in full force across the poultry industry. In addition, Virkon-S® were applied for prevention and decontamination procedures in the poultry house including the disinfection of surfaces, equipment, foot dips, vehicle wheel dips and cold fogging [3].

The present study shows that the blocking solution to neutralize the activity of PPMS resulted in almost no reduction of the

bacterial titer (P>0.05) between the positive control (tpc) and the tested solutions prior to adding the bacteria (0 sec). These results ensured that this blocking solution could stop the bacterial inactivity of PPMS, and apply this solution as an instrument for contact or exposure time determination. The current study is related to Sonthipet *et al*. [23] which used 0.7 M Tris-HCl and 30%FBS mixture for neutralizing PPMS, namely Lifejacket-T® (KBNP, Chungnam, Korea), including use of this blocking solution for exposure time determination.

Hence, food-borne diseases causing *E. coli* and *Salmonella* spp., are major bacterial diseases of zoonosis from poultry to human directly [3], so these bacteria were consumed as bacterial model for PPMS evaluation. The bactericidal efficacy indicated that PPMS could inactivate rif-SI, *E. coli* and SI, depending on the optimal concentration of PPMS, organic material conditions and exposure timing in the present study. The concentration, which is less than the manufacturing-recommended concentrations (1X), must extend the exposure time for bacterial inactivation. However, 1X until one-fourth (0.25X) of concentration, did not show a marked inactivation difference, even in both the absence and presence of organic material. Conversely, Jantafong *et al*. [8] reported that, with or without organic material conditions, a significant difference was demonstrated using a recommended concentration of QAC. Generally, QAC is a cationic detergent, which is a common disinfectant for animal farms and food processing industries, such as car gate sprays and foot baths. The advantage of QAC is its low toxicity and broad antimicrobial spectrum. However, the inactivation efficacies are usually reduced, even with organic material contamination [5, 7, 20]. Notwithstanding, the PPMS with and without organic material contamination did not show marked inactivation difference in the present study, indicating that PPMS more effective than QAC.

Generally, the purpose of disinfectant is to break down pathogens to reduce the risk of cross-contamination, especially bacteria and viruses. The present study, not only tested the efficacy of disinfectant using laboratory bacterial suspensions, but included the application on the artificial contaminated carrier surfaces, which compared the efficacy of both PPMS and QAC. Normally, bacteria, containing organic materials or cellular debris from infected animals, is excreted and firmly adheres to surface equipment around animals [12, 21]. The present study demonstrates that PPMS could inactivate bacteria which contaminates the surface of rubber, stainless steel and plastic to an undetectable level, using one-fourth (0.25X) of recommended concentrations within the lowest exposure time (30 sec). However, the efficacy of QAC could affect less than PPMS in the same conditions, especially on rubber surfaces, which could not inactivate bacteria within 5 min. These material models are commonly used in vehicle tyres, boots and tracks, including animal farm equipment, such as feeders, water pots, egg trays and chicken transport cages etc. Hence, the present study confirmed the efficacy and application of PPMS to inactivate bacteria on all carrier surfaces in the vicinity of animal farms.

The present study indicated that 54.45%PPMS could inactivate all bacteria such as rif-SI, *E. coli* and SI, and these result suggested that the bactericidal efficacy as same as other PPMS such as 21.41%PPMS (Virkon-S®, Dupont Animal Health Solutions, Wilmington, DE, USA) and 50%PPMS (Lifejacket-T®). In addition, its efficiency on various carrier surfaces, could be inactivated at a higher level than QAC. However, the bactericidal efficacies are depended on optimal concentration, organic material conditions and exposure/contact timing. Thus, this PPMS ensures its use as an alternative disinfectant, especially for biosecurity enhancement aiming to control bacteria that contaminates on and around animal farms.

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