SNRU Journal of Science and Technology

Journal home page: snrujst.snru.ac.th

Effects of Exposure to the Herbicides, Glyphosate and Paraquat, on the Growth Inhibition and Antibiotic Susceptibility of *Burkholderia pseudomallei*

Kanokporn Chaianunporn[1,*], Thotsapol Chaianunporn[2], Sorujsiri Chareonsudjai[3]
[1]Faculty of Medicine, Mahasarakham University, Maha Sarakham, 44000 Thailand
[2]Department of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen, 40002 Thailand
[3]Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002 Thailand
[*]**Corresponding Author**: kanokporn.s@msu.ac.th

Received: 4 Februar 2020; **Revised:** 10 April 2020; **Accepted:** 13 April 2020; **Available online:** 1 May 2020

Abstract

The emergence of antibiotic-resistant bacteria has increased due to selective pressure not just from antibiotics but also heavy metals, xenobiotic compounds and agrochemicals. Exposure to such compounds can induce genetic changes in bacteria which affect antibiotic susceptibility. In this study, we examined the effect of exposure to two herbicides, glyphosate and paraquat, on growth inhibition and antibiotic susceptibility of the soil bacterium *Burkholderia pseudomallei*. *B. pseudomallei* is the cause of a frequently fatal infectious disease, melioidosis, and antibiotic resistant strains of this species can cause severe clinical and public health problems. Our results show that glyphosate and paraquat inhibit *B. pseudomallei* growth, with median minimum inhibitory concentrations (MICs) of 3.00 ± 0.00% (w v^{-1}) for glyphosate and median MICs of 0.01 ± 0.00% (w v^{-1}) to 0.04 ± 0.00% (w v^{-1}) for paraquat. The MICs of ceftazidime (CAZ), doxycycline (DOX), trimethoprim (TMP), and sulfamethoxazole (SMX) against herbicide-treated and untreated *B. pseudomallei* were also determined. Glyphosate-treated and paraquat-treated *B. pseudomallei* were found to have decreased susceptibility to DOX and CAZ. Conversely, paraquat-treated *B. pseudomallei* became more susceptible to TMP. Taken together, these results show that exposure to glyphosate and paraquat inhibits *B. pseudomallei* growth and alters the bacterium's antibiotic response. These observations demonstrate the impact of herbicides on an environmental microorganism of medical importance.

Keywords: herbicides; antibiotic susceptibility; *Burkholderia pseudomallei*

1. Introduction

Antibiotic resistance is now recognized as one of the most serious threats to human health. Resistance to single and multiple antibiotics has been detected worldwide [1, 2]. Such resistant strains have caused severe clinical and public health problems. Initially, drug-resistant strains appeared in hospitals, where most antibiotics were being used [3]. However, antibiotic resistant bacteria are now emerging in the natural environment due to the use of antibiotics in humans, livestock and agriculture [1, 4, 5]. Moreover, the discharge of heavy metals, xenobiotic compounds, and organic solvents from industrial sites, mines and intensive farming is thought to be contributing to the environmental selection of antibiotic resistance in bacteria [6, 7].

The gram-negative bacillus *Burkholderia pseudomallei* is a species of bacteria found in both terrestrial and aquatic environments [8 – 10]. *B. pseudomallei* is the cause of a frequently fatal

infectious disease, melioidosis, which occurs in Southeast Asia and Australia [11 – 13]. Melioidosis is the third most common cause of infectious disease deaths in northeast Thailand. It is responsible for 20% of community acquired septicemias and has a 40% mortality rate [14]. Like other environmental bacteria, *B. pseudomallei* is inherently resistant to several antibiotics including all macrolides, all narrow-spectrum cephalosporins, most penicillins, all polymyxins and the aminoglycosides, leading to treatment difficulties [15 – 18]. Resistance can also be induced by environmental conditions. For example flooding and physical and chemical agricultural processes can induce genetic modifications in *B. pseudomallei* [13].

Nowadays, herbicides are intensively used in agriculture including rice cultivation where farmers are exposed to *B. pseudomallei* present in wet paddy field soil [10, 18, 19]. It has previously been reported that exposure to the herbicides dicamba, 2, 4-dichlorophenoxyacetic acid, and glyphosate can induce altered antibiotic susceptibility in *Escherichia coli* and *Salmonella* Typhimurium [20]. However, it has not yet been established whether herbicides affect antibiotic response in *B. pseudomallei*, an environmental pathogen commonly found in agricultural fields in northeast Thailand. The present study therefore examined the effects of sublethal concentrations of two widely used herbicides, glyphosate and paraquat, on antibiotic susceptibility in *B. pseudomallei*. The results may provide greater understanding of how antibiotic resistance is selected for in the natural environment.

2. Materials and Methods

Bacterial strains, herbicides, and antibiotics

B. pseudomallei strains K96243, 1026B, 38452 and 38457 were kindly provided by the Melioidosis Research Center at Khon Kaen University Faculty of Medicine. Herbicides were the commercial formulations gylphosate-48 (VIV Interchem Ltd., Thailand) containing 48% (w v^{-1}) glyphosate isopropylammonium, and paraquat (VIV Interchem Ltd., Thailand) containing 27.60% (w v^{-1}) 1, 1'-dimethyl-4, 4'bipyridinium dichloride. We tested the susceptibility of the *B. pseudomallei* strains above to four antibiotics, ceftazidime (CAZ, Sigma), doxycycline (DOX, Sigma), trimethoprim (TPM, Sigma), and sulfamethoxazone (SMX, Sigma). Test antibiotics were selected based on their clinical use to treat *B. pseudomallei* infection [15]. Stock solutions of antibiotics were stored at –20 °C.

Bacterial inoculum preparation

Each strain of *B. pseudomallei* was sub-cultured at 37 ºC for 48 h on Ashdown's agar. A single bacterial colony was selected, subsequently suspended in Trypticase soy broth (TSB), and incubated at 37 ºC for 3 h. After that, bacterial cultures were harvested and re-suspended in Mueller-Hinton broth (MHB) and adjusted to 0.50 McFarland standards (approximately 10^8 CFU ml^{-1}) [21].

Bacteria cultured under stress conditions

To induce stress conditions, herbicides at sub-lethal concentration were added into culture media. In brief, 10^8 CFU ml^{-1} of *B. pseudomallei* were cultured in TSB with 0.30% (w v^{-1}) glyphosate or 0.001% (w v^{-1}) paraquat at 37 °C for 7 days in a rotary shaker [20, 22]. Control bacteria were grown under the same conditions but without these herbicides. All cultured bacteria were subsequently harvested, re-suspended in MHB and adjusted to 10^6 CFU ml^{-1} [21] .

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of herbicides and antibiotics

MICs of the herbicides and antibiotics were determined using the broth microdilution method [23]. Two-fold serial dilutions of the herbicides or antibiotics were prepared. The final concentrations ranged from 24 to 0.01% (w v^{-1}) for glyphosate, 2.76 to 0.001% (w v^{-1}) for paraquat, 4000 to 19.50 µg ml^{-1}

for SMX, and 1000 to 0.48 μg ml^{-1} for CAZ, DOX and TMP. Ninety-six-well plates were prepared containing 50 μl of 2-fold dilution concentrations of the herbicides or antibiotics in MHB. Each well was then inoculated with 50 μl of 10^6 CFU ml^{-1} bacterial suspension and incubated at 37 °C for 16 – 18 h. Tetracycline (Tet) at a concentration of 512 μg ml^{-1} and MHB were used as positive and negative controls, respectively. The MICs of the antibiotics was determined using resazurin indicator solution. The lowest concentration to cause the resazurin to change from purple to pink or colorless was taken as the MIC value. Because herbicides interfere with the color of resazurin, the MICs of glyphosate and paraquat was determined by identifying the lowest concentration at which the suspension remained as clear as the positive control. All experiments were performed in triplicate.

To determine the MBC, a loopful of suspension from all of the wells that remained free from visible growth or color change was inoculated onto nutrient agar (NA) and incubated at 37 °C for 24 h. After incubation, the minimum concentration at which no visible colonies were detected was recorded as the MBC [21].

Statistical analysis

MICs and MBCs were expressed as median and interquartile ranges (IQR) as the data is non-normally distributed. The Kruskal-Wallis test was used to compare results for the same bacterial strain cultured under the three different conditions: control, sublethal concentration of glyphosate and sublethal concentration of paraquat. If the Kruskal-Wallis test detected a significant difference between control and treatments (p-value<0.05), we further compared each pair with the Mann-Whitney U-test. As in the Kruskal-Wallis test, a p-value below 0.05 was considered significant in the Mann-Whitney U-test.

3. Results and Discussion

MICs and MBCs of glyphosate and paraquat against B. pseudomallei

Glyphosate was found to have an MIC of 3.00% (w v^{-1}) and an MBC of 12.00% (w v^{-1}) against *B. pseudomallei* (Table 1). Glyphosate is known to kill bacteria by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the Shikimate pathway. This enzyme is crucial for the biosynthesis of aromatic amino acids, and its inhibition disrupts tyrosine, phenylalanine and tryptophan synthesis [24 – 28]. This enzyme is present in *B. pseudomallei* [28].

For paraquat, the MIC values ranged from 0.01 to 0.04% (w v^{-1}) and the MBC values ranged from 0.02 to 2.76% (w v^{-1}) for *B. pseudomallei* (Table 1). This finding shows that the four test strains of *B. pseudomallei* vary in their susceptibility to paraquat (Table 1). Paraquat is an oxidant that interferes with electron transfer. It accepts electrons from an electron donor such as NADPH and transfers them to oxygen. Therefore, destructive reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals are produced. These ROS molecules damage several bacterial components including membranes, proteins and nucleic acids [29, 30]. Some bacteria can avoid ROS-mediated killing by producing enzymes called superoxide dismutases (SodA, SodB and SodC) [30 – 34] and this may explain the variation in *B. pseudomallei* susceptibility to paraquat. Variation in paraquat susceptibility might also be due to differences in the abilities of the *B. pseudomallei* strains to generate biofilms, since biofilm formation enables bacteria to survive in the presence of antimicrobial agents and ROS [35, 36].

Table 1 MICs and MBCs of glyphosate and paraquat against *B. pseudomallei* (Bp)

Strain of Bp	Glyphosate		Paraquat	
	MIC [% (w v^{-1})]	MBC [% (w v^{-1})]	MIC [% (w v^{-1})]	MBC [% (w v^{-1})]
K96243	3.00 ± 0.00	12.00 ± 6.00	0.01 ± 0.00	0.69 ± 0.67
1026B	3.00 ± 0.00	12.00 ± 6.00	0.02 ± 0.01	0.69 ± 0.65
38452	3.00 ± 0.00	12.00 ± 13.50	0.04 ± 0.08	2.76 ± 2.75
38457	3.00 ± 0.00	12.00 ± 9.00	0.01 ± 0.00	0.02 ± 0.04

Data are expressed as Median ± IQR

Herbicide-induced antibiotic response alteration

Changes in antibiotic susceptibility (MICs and/or MBCs) of the bacteria following exposure to a sublethal dose of two herbicides (stress conditions) were evaluated. No difference was detected in the MICs or MBCs of CAZ between control and treatment groups in any of the test strains of *B. pseudomallei* except *B. pseudomallei* strain K96243. In *B. pseudomallei* strain K96243, paraquat significantly increased the MBC of CAZ (Kruskal-Wallis rank sum test, chi-squared = 7.0351, df = 2, p-value = 0.030) (Table 2). Moreover, a significant increase in the MIC of DOX was observed when *B. pseudomallei* strain 38457 was cultured in the presence of glyphosate or paraquat (Kruskal-Wallis rank sum test, chi-squared = 8, df = 2, p-value = 0.018) (Table 2). We also observed a decrease in the MIC of TMP when strain 38457 was cultured in the presence of paraquat (Kruskal-Wallis rank sum test, chi-squared = 7.624, df = 2, p-value = 0.022) (Table 2). No statistically significant difference was detected in the MICs or MBCs of SMX following herbicide exposure in any of the test strains of *B. pseudomallei* (Table 2).

Table 2 MICs and MBCs of antibiotics against *B. pseudomallei* (Bp) cultured in the absence (control) or presence of herbicides (stress conditions)

Strain of Bp	Antibiotics	Control		0.3%(w v^{-1}) Glyphosate		0.001% (w v^{-1}) Paraquat	
		MIC (μg ml^{-1})	MBC (μg ml^{-1})	MIC (μg ml^{-1})	MBC (μg ml^{-1})	MIC (μg ml^{-1})	MBC (μg ml^{-1})
K96243	CAZ	1.95 ± 0.00	7.81 ± 0.00	1.95 ± 0.00	7.81 ± 0.00	1.95 ± 0.00	93.75 ±3 9.06*
	DOX	1.95 ± 0.00	250 ± 0.00	1.95 ± 0.00	500 ± 0.00	1.95 ± 0.00	500 ± 0.00
	TMP	7.81 ± 1.95	125 ± 0.00	7.81 ± 0.00	125 ± 0.00	7.81 ± 0.00	> 500
	SMX	125 ± 0.00	> 2,000	125 ± 0.00	2,000 ± 0.00	125 ± 0.00	> 2,000
1026B	CAZ	1.95 ± 0.00	15.63 ± 3.91	2.93 ± 0.98	62.5 ± 23.44	3.91 ± 0.49	250 ± 62.50
	DOX	0.24 ± 0.00	125 ± 31.25	0.24 ± 0.00	125 ± 0.00	0.24 ± 0.00	125.00 ± 0.00
	TMP	0.49 ± 0.00	62.50 ± 15.63	0.49 ± 0.00	31.25 ± 0.00	0.49 ± 0.00	31.25 ± 0.00
	SMX	31.25 ± 0.00	1,000 ± 0.00	31.25 ± 7.81	> 1,000	31.25 ± 7.81	1,000 ± 250.00
38452	CAZ	2.93 ± 0.98	7.81 ± 0.00	3.91 ± 0.49	11.72 ± 3.91	2.93 ± 0.98	11.72 ± 3.91
	DOX	0.49 ± 0.00	31.25 ± 11.72	0.49 ± 0.00	15.63 ± 0.00	0.49 ± 0.00	3.91 ± 0.00
	TMP	1.95 ± 0.00	125 ± 0.00	1.95 ± 0.00	125 ± 0.00	1.95 ± 0.00	> 500
	SMX	15.63 ± 0.00	1,000 ± 0.00	15.63 ± 3.91	1,000 ± 0.00	15.63 ± 0.00	1,000 ± 0.00
38457	CAZ	3.91 ± 0.49	5.86 ± 1.95	2.93 ± 0.98	15.63 ± 5.86	3.91 ± 0.98	11.72 ± 4.88
	DOX	0.49 ± 0.00	187.50 ± 31.25	0.98 ± 0.00*	250 ± 0.00	0.98 ± 0.00*	93.75 ± 15.63
	TMP	15.63 ± 0.00	125 ± 0.00	15.63 ± 0.00	125 ± 0.00	1.95 ± 0.49*	62.50 ± 0.00
	SMX	31.25 ± 0.00	250 ± 0.00	31.25 ± 7.81	250 ± 0.00	15.63 ± 3.91	250.00 ± 0.00

Data were expressed as Median ± IQR, Asterisk (*) indicates $P < 0.05$ (Kruskal-Wallis test – Mann-Whitney U-test.

Antibiotic susceptibility following exposure to sublethal concentrations of herbicide varied between the bacterial strains. Exposure to glyphosate or paraquat significantly decreased the susceptibility of *B. pseudomallei* strains K96243 and 38457 to CAZ and DOX, whilst simultaneously increasing the susceptibility of strain 38457 to TMP. Our findings agree with previous reports that there are significant differences in the biocide susceptibility of different strains of the same bacterial

species [20, 37, 38]. Herbicide-induced changes in antibiotic susceptibility may be due to increases or decreases in the expression of efflux pumps [39]. Previous studies have shown, for example, that the AcrAB-TolC efflux pump is upregulated when *E. coli* and *S.* Typhimurium are exposed to toxic chemicals. This results in reduced susceptibility to several drugs including those in the tetracycline class [20, 40, 41]. In our study, we expected that glyphosate and / or paraquat would also increase the number of efflux pumps present at the bacterial cell membrane, thereby decreasing bacterial susceptibility to the test antibiotics. However, in many cases the herbicide-treated bacteria were more antibiotic-sensitive than the untreated control bacteria. This might be due to the herbicide glyphosate hindering amino acid synthesis or due to paraquat producing reactive oxygen species, with these events weakening the bacterial cells and leaving them more antibiotic-sensitive.

4. Conclusion

Herbicides are applied to agricultural crops to improve pre-harvest desiccation [42], but residual herbicide then remains in the environment and may affect microbial communities [20, 43, 44]. In this study, we demonstrated the inhibitory effects of the herbicides, glyphosate and paraquat, on *B. pseudomallei* and how exposure to sublethal concentrations of these herbicides can affect bacterial susceptibility to antibiotics. In the natural environment, for example in soil and in water, antibiotics may not be present in sufficient quantities to give bacteria with low-level resistance a competitive advantage [45]. However, herbicide-induced changes in antibiotic MIC could be problematic if this alteration in MIC is sufficient to make low levels of endogenous antibiotics and pollutants a relevant selective force [20, 46]. Although we did not see a dramatic change in antibiotic susceptibility following bacterial exposure to the two herbicides in this study, the changes in MIC we did detect are still of concern. Slight increases in antibiotic MIC resulting in low-level resistance can compromise therapy [47]. Not all treatment failures result from bacteria with full resistance genotypes; transient, induced, higher MICs may also contribute to these failures [48, 49]. Moreover, in healthcare settings, transient increases in MIC significantly increase the probability of spontaneous mutations, leading to transmission of low-level resistance and perhaps additional changes conferring high level resistance [20, 50, 51]. It is thus important that we understand the effects of these herbicides on pathogenic bacteria, especially herbicide-induced changes in antibiotic resistance.

5. Suggestions

In this study, we found that glyphosate and paraquat could inhibit *B. pseudomallei*. However, the effects of these two herbicides on *B. pseudomallei* in the natural environment might differ from our *in vitro* study because these herbicides possibly affect other bacterial species. Herbicide-induced changes to bacterial communities in the soil could favor pathogens such as *B. pseudomallei*, increasing the risk of infection for agricultural workers. At the present time, little knowledge is available regarding the effect of glyphosate and paraquat residues on bacterial communities. Further studies are therefore warranted to determine if herbicides exert a selective pressure on bacterial communities.

6. Acknowledgements

This study was funded by a grant from Mahasarakham University (MSU) Faculty of Medicine. The authors would like to acknowledge the Melioidosis Research Center, Khon Kaen University (KKU), Thailand for providing the *B. pseudomallei* strains. We are grateful to KKU Faculty of Medicine and KKU Faculty of Science for provision of laboratory space and equipment. Lastly, we would also like to thank MSU Faculty of Medicine for language-editing assistance.

7. References

[1] S.B. Levy, B. Marshall, Antibacterial resistance worldwide: causes, challenges and responses, Nat. Med. 10(S12) (2004) S122 – 129.

[2] Y.-Y. Liu, Y. Wang, T.R. Walsh, L.-X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L.-F. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J.-H. Liu, J. Shen, Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study, Lancet Infect. Dis. 16(2) (2016) 161 – 168.

[3] S.B. Levy, The Challenge of Antibiotic Resistance, Sci. Am. 278(3) (1998) 46 – 53.

[4] B.M. Marshall, S.B. Levy, Food animals and antimicrobials: impacts on human health, Clin. Microbiol. Rev. 24(4) (2011) 718 – 733.

[5] K. Chaianunporn, S. Tanuchit, S. Thammawat, T. Chaianunporn, Antibiotic resistance of environmental Isolates of *Pseudomonas aeruginosa* in Maha Sarakham province and Nong Bua Lamphu province, J. Sci. Technol. MSU. 35(2) (2016) 174 – 181.

[6] A. Alonso, P. Sanchez, J.L. Martinez, Environmental selection of antibiotic resistance genes. Minireview, Environ. Microbiol. 3(1) (2001) 1 – 9.

[7] K. Rangasamy, M. Athiappan, N. Devarajan, G. Samykannu, J.A. Parray, K.N. Aruljothi, N. Shameem, A.A. Alqarawi, A. Hashem, E.F. Abd_Allah, Pesticide degrading natural multidrug resistance bacterial flora, Microb. Pathog. 114 (2018) 304 – 310.

[8] A.C. Cheng, B.J. Currie, Melioidosis: epidemiology, pathophysiology, and management, Clin. Microbiol. Rev. 18(2) (2005) 383 – 416.

[9] D. Limmathurotsakul, S. Wongratanacheewin, N.P.J. Day, N. Teerawattanasook, W. Chaowagul, S. Chaisuksant, P. Chetchotisakd, S.J. Peacock, G. Wongsuvan, Increasing incidence of human melioidosis in northeast Thailand, Am. J. Trop. Med. Hyg. 82(6) (2010) 1113 – 1117.

[10] S. Rattanavong, V. Wuthiekanun, S. Langla, P. Amornchai, J. Sirisouk, R. Phetsouvanh, C.E. Moore, S.J. Peacock, Y. Buisson, P.N. Newton, Randomized soil survey of the distribution of *Burkholderia pseudomallei* in rice fields in Laos, Appl. Environ. Microbiol. 77(2) (2011) 532 – 536.

[11] B.J. Currie, D.A.B. Dance, A.C. Cheng, The global distribution of *Burkholderia pseudomallei* and melioidosis: an update, Trans. R. Soc. Trop. Med. Hyg. 102 (2008) S1 – 4.

[12] S. Hinjoy, V. Hantrakun, S. Kongyu, J. Kaewrakmuk, T. Wangrangsimakul, S. Jitsuronk, W. Saengchun, S. Bhengsri, T. Akarachotpong, S. Thamthitiwat, O. Sangwichian, S. Anunnatsiri, R.W. Sermswan, G. Lertmemongkolchai, C.S. Tharinjaroen, K. Preechasuth, R. Udpaun, P. Chuensombut, N. Waranyasirikul, C. Anudit, S. Narenpitak, Y. Jutrakul, P. Teparrukkul, N. Teerawattanasook, K. Thanvisej, A. Suphan, P. Sukbut, K. Ploddi, P. Sirichotirat, B. Chiewchanyon, K. Rukseree, M. Hongsuwan, G. Wongsuwan, P. Sunthornsut, V. Wuthiekanun, S. Sachaphimukh, P. Wannapinij, W. Chierakul, C. Chewapreecha, J. Thaipadungpanit, N. Chantratita, S. Korbsrisate, A. Taunyok, S. Dunachie, P. Palittapongarnpim, S. Sirisinha, R. Kitphati, S. Iamsirithaworn, W. Chaowagul, P. Chetchotisak, T. Whistler, S. Wongratanacheewin, D. Limmathurotsakul, Melioidosis in Thailand: present and future, Trop. Med. Infect. Dis. 3(2) (2018) 38.

[13] V. Wuthiekanun, D. Limmathurotsakul, N. Chantratita, E.J. Feil, N.P.J. Day, S.J. Peacock, *Burkholderia pseudomallei* is genetically diverse in agricultural land in northeast Thailand, PLoS Negl. Trop. Dis. 3(8) (2009) e496.

[14] W.J. Wiersinga, B.J. Currie, S.J. Peacock, Melioidosis, N. Engl. J. Med. 367(11) (2012) 1035 – 1044.

[15] T.J.J. Inglis, The treatment of melioidosis, Pharmaceuticals. 3(5) (2010) 1296 – 1303.

[16] K.A. Rhodes, H.P. Schweizer, Antibiotic resistance in *Burkholderia* species, Drug Resist. Updat. 28 (2016) 82 – 90.

[17] H.P. Schweizer, Mechanisms of antibiotic resistance in *Burkholderia pseudomallei*: implications for treatment of melioidosis, Future Microbiol. 7(12) (2012) 1389 – 1399.

[18] V. Wuthiekanun, P. Amornchai, N. Saiprom, N. Chantratita, W. Chierakul, G.C.K.W. Koh, W. Chaowagul, N.P.J. Day, D. Limmathurotsakul, S.J. Peacock, Survey of antimicrobial resistance in clinical *Burkholderia pseudomallei* isolates over two decades in northeast Thailand, Antimicrob. Agents Chemother. 55(11) (2011) 5388 – 5391.
[19] W. Chaowagul, N.J. White, D.A.B. Dance, Y. Wattanagoon, P. Naigowit, T.M.E. Davis, S. Looareesuwan, N. Pitakwatchara, Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand, J. Infect. Dis. 159(5) (1989) 890 – 899.
[20] B. Kurenbach, D. Marjoshi, C.F. Amábile-Cuevas, G.C. Ferguson, W. Godsoe, P. Gibson, J.A. Heinemann, Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, MBio. 6(2) (2015) e00009 – 15.
[21] K. Chaianunporn, W. Chatuphonprasert, W. Mongkolthanaruk, T. Chaianunporn, Phytochemical analysis and effect of *Senna alata* leaf extract fractions on methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, J. Sci. Technol. MSU. 37(2) (2018) 180 – 190.
[22] B.Y. Reeks, F.R. Champlin, D.B. Paulsen, D.W. Scruggs, M.L. Lawrence, Effects of sub-minimum inhibitory concentration antibiotic levels and temperature on growth kinetics and outer membrane protein expression in *Mannheimia haemolytica* and *Haemophilus somnus*, Can. J. Vet. Res. Rev. Can. Rech. Veterinaire. 69(1) (2005) 1 – 10.
[23] S.D. Sarker, L. Nahar, Y. Kumarasamy, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, Methods. 42(4) (2007) 321 – 324.
[24] R.S. Fischer, A. Berry, C.G. Gaines, R.A. Jensen, Comparative action of glyphosate as a trigger of energy drain in eubacteria., J. Bacteriol. 168(3) (1986) 1147 – 1154.
[25] R.M. Zablotowicz, K.N. Reddy, Impact of glyphosate on the symbiosis with glyphosate-resistant transgenic soybean, J. Environ. Qual. 33(3) (2004) 825 – 31.
[26] M.H. Norris, Y. Kang, D. Lu, B.A. Wilcox, T.T. Hoang, Glyphosate resistance as a novel select-agent-compliant, non-antibiotic-selectable marker in chromosomal mutagenesis of the essential genes asd and dapB of *Burkholderia pseudomallei*, Appl. Environ. Microbiol. 75(19) (2009) 6062 – 6075.
[27] J.M. Staub, L. Brand, M. Tran, Y. Kong, S.G. Rogers, Bacterial glyphosate resistance conferred by overexpression of an *E. coli* membrane efflux transporter, J. Ind. Microbiol. Biotechnol. 39(4) (2012) 641 – 647.
[28] M. Kanehisa, M. Furumichi, M. Tanabe, Y. Sato, K. Morishima, KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res. 45(D1) (2017) D353 – 361.
[29] J.A. Imlay, Pathways of oxidative damage, Annu. Rev. Microbiol. 57(1) (2003) 395 – 418.
[30] M. Vanaporn, M. Wand, S.L. Michell, M. Sarkar-Tyson, P. Ireland, S. Goldman, C. Kewcharoenwong, D. Rinchai, G. Lertmemongkolchai, R.W. Titball, Superoxide dismutase C is required for intracellular survival and virulence of *Burkholderia pseudomallei*, Microbiology. 157(8) (2011) 2392 – 2400.
[31] S.C. Grace, Phylogenetic distribution of superoxide dismutase supports an endosymbiotic origin for chloroplasts and mitochondria, Life Sci. 47(21) (1990) 1875 – 1886.
[32] K.L.R. Dunn, J.L. Farrant, P.R. Langford, J.S. Kroll, Bacterial [Cu,Zn]-cofactored superoxide dismutase protects opsonized, encapsulated *Neisseria meningitidis* from phagocytosis by human monocytes/macrophages, Infect. Immun. 71(3) (2003) 1604 – 1607.
[33] I.-H. Kang, J.S. Kim, J.K. Lee, The virulence of *Vibrio vulnificus* is affected by the cellular level of superoxide dismutase activity, J. Microbiol. Biotechnol. 17(8) (2007) 1399 – 1402.
[34] K.E. Keith, M.A. Valvano, Characterization of SodC, a periplasmic superoxide dismutase from *Burkholderia cenocepacia*, Infect. Immun. 75(5) (2007) 2451 – 2460.

[35] M.R. Parsek, P.K. Singh, Bacterial biofilms: an emerging link to disease pathogenesis, Annu. Rev. Microbiol. 57 (2003) 677 – 701.
[36] H.C. Flemming, J. Wingender, The biofilm matrix, Nat. Rev. Microbiol. 8(9) (2010) 623 – 633.
[37] J.L. Rosner, Nonheritable resistance to chloramphenicol and other antibiotics induced by salicylates and other chemotactic repellents in *Escherichia coli* K-12., Proc. Natl. Acad. Sci. 82(24) (1985) 8771 – 8774.
[38] W.H. Wang, Aspirin inhibits the growth of *Helicobacter pylori* and enhances its susceptibility to antimicrobial agents, Gut. 52(4) (2003) 490 – 495.
[39] D. Du, Z. Wang, N.R. James, J.E. Voss, E. Klimont, T. Ohene-Agyei, H. Venter, W. Chiu, B.F. Luisi, Structure of the AcrAB–TolC multidrug efflux pump, Nature. 509(7501) (2014) 512 – 515.
[40] M.N. Alekshun, S.B. Levy, The mar regulon: multiple resistance to antibiotics and other toxic chemicals, Trends Microbiol. 7(10) (1999) 410 – 413.
[41] L. Fernandez, R.E.W. Hancock, Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance, Clin. Microbiol. Rev. 25(4) (2012) 661 – 681.
[42] C. Bellé, S.M. Kulczynski, C.J. Basso, T. Edu Kaspary, F.P. Lamego, M.A.B. Pinto, Yield and quality of wheat seeds as a function of desiccation stages and herbicides, J. Seed Sci. 36(1) (2014) 63 – 70.
[43] J.A.S. Moretto, L.M. Altarugio, P.A. Andrade, A.L. Fachin, F.D. Andreote, E.G. Stehling, Changes in bacterial community after application of three different herbicides, FEMS Microbiol. Lett. 364(13) (2017). 1 – 6.
[44] C. Thiour-Mauprivez, F. Martin-Laurent, C. Calvayrac, L. Barthelmebs, Effects of herbicide on non-target microorganisms: towards a new class of biomarkers?, Sci. Total Environ. 684 (2019) 314 – 325.
[45] C.F. Amábile-Cuevas, Antibiotic resistance: from Darwin to Lederberg to Keynes, Microb. Drug Resist. 19(2) (2013) 73 – 87.
[46] F. Mauffrey, P.Y. Baccara, C. Gruffaz, S. Vuilleumier, G. Imfeld, Bacterial community composition and genes for herbicide degradation in a stormwater wetland collecting herbicide runoff, Water. Air. Soil Pollut. 228(12) (2017) 452.
[47] M. Haeseker, L. Stol,, F. Nieman, C. Hoebe, C. Neef, C. Bruggeman, A. Verbon, The ciprofloxacin target AUC : MIC ratio is not reached in hospitalized patients with the recommended dosing regimens: ciprofloxacin concentrations in hospitalized patients, Br. J. Clin. Pharmacol. 75(1) (2013) 180 – 185.
[48] A. Forrest, D.E. Nix, C.H. Ballow, T.F. Goss, M.C. Birmingham, J.J. Schentag, Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients., Antimicrob. Agents Chemother. 37(5) (1993) 1073 – 1081.
[49] R.J. Hassing, W.H.F. Goessens, D.J. Mevius, W. Pelt, J.W. Mouton, A. Verbon, P.J. Genderen, Decreased ciprofloxacin susceptibility in *Salmonella* Typhi and Paratyphi infections in ill-returned travellers: the impact on clinical outcome and future treatment options, Eur. J. Clin. Microbiol. Infect. Dis. 32(10) (2013) 1295 – 1301.
[50] F. Baquero, Low-level antibacterial resistance: a gateway to clinical resistance, Drug Resist. Updat. 4(2) (2001) 93 – 105.
[51] Z. Shen, X.Y. Pu, Q. Zhang, Salicylate functions as an efflux pump inducer and promotes the emergence of fluoroquinolone-resistant *Campylobacter jejuni* mutants, Appl. Environ. Microbiol. 77(20) (2011) 7128 – 7133.