

KNOWLEDGE ON MAINTAINING COLD CHAIN FOR CHILDHOOD IMMUNISATION VACCINES AT THE PRIMARY HEALTHCARE SETTING MALAYSIA

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ABSTRACT

Background: A proper maintenance of vaccine cold chain is important to achieve full benefit of childhood immunisation, apart from high remarkable childhood immunisation coverage and timely administration of vaccines. Ensuring an adequate knowledge among healthcare workers in maintaining vaccine cold chain system is crucial to ensure the efficacy of vaccines being administered and the effectiveness of the national immunisation programme.

Objective: To determine the contributing factors towards knowledge related to vaccine cold chain maintenance among private healthcare assistants at primary care level in the district of Hulu Langat, Selangor.

Materials and Methods: A cross sectional study was conducted from 1st February until 30th July 2016 among 493 healthcare assistants from 270 private clinics, which were randomly selected in Hulu Langat District, Selangor. A validated self-administered questionnaire was used which consisted of five sections on sociodemographic, duration of working experience in healthcare field, history of training related to maintaining vaccine cold chain, attitude towards maintaining vaccine cold chain and knowledge on maintaining vaccine cold chain. Data collected was analysed using IBM Statistical Package for Social Science (SPSS) version 22.0. Three stages of analysis were conducted; descriptive analysis (mean and median), bivariate analysis (Chi square (χ 2) or Fisher Exact Test) and multivariate analysis (multiple logistic regression). In this study, *P* value of <0.05 was considered statistically significant.

Result: The response rate was 91.3%. Only 235 (47.7%) respondents had good knowledge on vaccine cold chain. It was found that, there was a significant association between knowledge on maintaining vaccine cold chain and race (χ^2 =6.016, P=0.013), monthly income (χ^2 =12.024, P=0.007), duration of working experience in healthcare field (χ^2 =8.429, P=0.052), history of attending training related to maintaining vaccine cold chain (χ^2 =29.884, P=0.001) and attitude towards maintaining vaccine cold chain (χ^2 =86.087, χ^2 =0.001). Significant predictors for good knowledge on maintaining vaccine were; monthly income more than RM 2001 and above (AOR =4.50, 95% CI [1.410, 14.353]), history of attending training (AOR=3.25, 95% CI [1.796, 5.868]) and positive attitude towards maintaining vaccine cold chain (AOR=5.82, 95% CI [3.841, 8.8821]).



Conclusion: The finding of this study indicate poor level of knowledge related to maintaining vaccine cold chain among private healthcare assistants in Hulu Langat District, Selangor with attitude towards maintaining vaccine cold chain being the most significant predictor towards good knowledge. Initiatives related to quality improvement activities in order to improve knowledge on maintaining vaccine cold chain should be properly planned which include continuous training and supervision as well as ensuring availability of educational materials may improve knowledge related to the maintenance of vaccine cold chain.

Keywords: vaccine, cold chain, healthcare assistants, immunisation, primary care

1.0 Introduction

Immunisation is the most cost effective public health investment in disease prevention. It benefits all people, not only through improvements in health and life expectancy but also through its social and economic impact at the global, national and community level. It greatly reduces the incidences of vaccine-preventable diseases, disability, and death worldwide (Andre, Booy, Bock, Clemens & Datta, 2008). Despite the remarkable achievement and massive coverage, outbreaks and increasing trend of re-emerging of vaccine-preventable diseases such as pertussis and measles have been reported. It is estimated that 17% of all death among children under five is still related to vaccine preventable disease (WHO, 2014).

Three main factors to achieve full benefit of immunisation include; wide immunisation coverage, timely administration of vaccines and ensuring potency of vaccines through proper maintenance of vaccine cold chain. The "cold chain" refers to the continuum of safe handling practices, including materials, equipment and procedures, that maintain vaccines in between 2°C and 8°C to maintain their potency from the time they are manufactured to the time they are administered to patients (Weir & Hatch, 2004). Basic components that determine the effectiveness of vaccine cold chain system include; transport and storage equipment, efficient management procedures and trained personal (Rogers, Dennison, Adepoju, Dowd & Uedoi, 2010).

Healthcare providers must possess knowledge and skills on managing cold chain as they are part of the main element in maintaining vaccine cold chain. Knowledge on maintaining vaccine cold chain should include proper vaccine storage and handling procedures, equipment maintenance and repair procedures, appropriate action to be taken in the event of a vaccine exposure and contingency plans and ensure that they are in place in the event of premises closure during staff vacation, equipment failure and or electrical disruptions (Public Health Unit Ontario, 2013). Several studies have shown that knowledge of good vaccine operation and cold chain system among healthcare workers in maintaining the vaccine cold chain are inadequate (Yuan, Daniels, Naus & Brcic ,1995; Grasso, 1999; Naik, Rupani & Bansal, 2013; Oliveira, Gallardo, Maria, Cavalcante & Ricardo et al, 2015)

In Malaysia, primary healthcare is provided by both public and private healthcare providers in which routine child immunisation is part of the service provided by both sectors. According to report from Ministry of Health (MOH) in 2014, private health care contributed approximately



10% vaccination uptake in Malaysia. Although the number seems to be small, its contribution may have a significant impact in maintaining herd immunity in a population. Little information is available regarding the extent to which the private health care assistants in primary care level meet the quality assurance required to maintain vaccine cold chain or having the necessary equipment though they also play an important role as immunisation providers. Hence, a study on the knowledge of private healthcare assistants on vaccine cold chain maintenance is a necessity.

Until August 2015, Selangor State Health Department reported that Hulu Langat District has the highest reported cases of measles (39.5%) and pertussis (20.95%) cases among children compared to other districts in Selangor. Therefore, Hulu Langat District was chosen for the purpose of this study to evaluate the knowledge of private healthcare assistants in maintaining vaccine cold chain.

2.0 Materials and Methods

A cross sectional study was conducted between February and July 2016. Cluster sampling method was chosen with private clinics in the district of Hulu Langat as the cluster. A total of 270 private clinics were randomly selected using table of random numbers from 480 private clinics in Hulu Langat District, Selangor, registered under Private Medical Practice Control Branch, Medical Practice Division, MOH. All private healthcare assistants present in the selected health clinic during data collection were included in the study.

Validated self-administered questionnaires were distributed to all private healthcare assistants working at selected private health clinics. The validated questionnaire consists of 5 sections namely, socio-demographic factors, duration of working experience in healthcare field, history of attending training related to the maintenance of vaccine cold chain, knowledge on vaccine cold chain maintenance and attitude toward maintenance of vaccine cold chain. The cut-off level for knowledge and attitude was determined by the mean value of total score of each item respectively; good knowledge ($\geq 75.0\%$), poor knowledge (< 75.0%) and positive attitude ($\geq 77.0\%$), negative attitude (< 77.0%).

Data collected was analysed using IBM Statistical Package for Social Science (SPSS) version 22.0. For data analysis, all continuous variables were described as median [Interquartile Range (IQR)] or means [standard deviations (SD)]. Categorical data were recorded as frequency (n) and percentages (%). In terms of data analysis, Chi square (χ 2) or Fisher Exact Test was used to measure for association. Multivariate logistic regression was used to determine predictors. P value of < 0.05 was considered statistically significant.

3.0 Result

3.1 Characteristics of respondents

Table 1 shows the characteristics of the respondents. Age of the respondents was collected as continuous data. Median age for the overall respondents was 26 (IQR= 7) years old. Majority



of the respondents were female (98.6%), Malay (74.0%), highest level of education up to Diploma level and above (55.6%), single 59.4% and having less than 3 number of children (89.7%), working in healthcare field more than 12 months (66.3%) and never attended any training related to vaccine cold chain (82.8%).

Table 3.1: Characteristics of respondents (N=493)

Characteristics	Median	n	%
Age (Years)	26 (IQR =7)		
Gender			
Male		7	1.4
Female		486	98.6
Ethnicity			
Malay		365	74.0
Chinese		43	8.7
Indian		71	15.0
Others		11	2.2
Level of Education			
Primary school		0.0	0.0
Secondary school		219	44.4
Diploma and above		274	55.6
Marital Status			
Single		293	59.4
Married		200	39.1
Number of children			
<3		442	89.7
3-4		45	9.1
>5		6	1.2
Duration of working			
experience in			
healthcare field			
3-6months		96	19.5
6-12 months		70	14.2
≥12months		327	66.3
History of attending			
training			
Yes		85	17.2
No		408	82.8

3.2 Characteristics of respondents according to knowledge and attitude (N=493)

Table 2 shows the distribution of total score of knowledge and attitude on maintaining vaccine cold chain. For knowledge, a total of 258 (52.3%) of the respondents have poor knowledge with score above mean value as compared to another 235(47.7%) respondents with good knowledge on maintaining vaccine cold chain. Meanwhile for attitude, a total of 215 (43.6%) respondents with positive attitude as compared to another 278 (56.4%) of the respondents were having negative attitude toward maintaining vaccine cold chain.



Table 2: Characteristics of respondents according to knowledge and attitude (N=493)

Variable	n	%
Knowledge (Mean = 74.63 ± 9.70)		
Good (≥ 75.0)	235	47.7
Poor (<75.0)	258	52.3
Attitude (Mean=77.08±11.91)		
Positive (≥77.0)	215	43.6
Negative (<77.0)	278	56.4

3.3 Factors associated with knowledge on maintaining vaccine cold chain

Table 3 shows factors associated between knowledge on maintaining vaccine cold chain. Among all factors listed in the table, there were five factors that have significant association with knowledge on maintaining vaccine cold chain; race (χ^2 =6.106, df=1, P<0.05) and monthly income (χ^2 =12.024, df=3, P<0.05), duration of working experience in healthcare field (χ^2 =8.429, df=2, P<0.05), history of attending training related to vaccine cold chain (χ^2 =29.884, df=1, P<0.05) and attitude toward maintaining vaccine cold chain (χ^2 =86.067, df=1, df=1,

Table 3: Factors associated with knowledge on maintaining vaccine cold chain (N=493)

Factor	Knowledge		X ² /Fisher	df	P value
	Good	Poor	exact test		
	n (%)	n (%)			
Age (Median = 26 (IQR)=7)					
<26 years old	103 (49.0)	107(51.0)			
≥26 years old	132 (46.6)	151(53.4)	0.279	1	0.597^{a}
Gender					
Male	4 (57.1)	3 (42.9)	0.066	1	$0.798^{\rm b}$
Female	232 (47.7)	254 (52.3)			
Race					
Malay	186 (51.0)	179(49.0)	6.106	1	0.013^{a^*}
Non Malay	49(20.9)	186(79.1)			
Highest level of					
Education					
< Tertiary level	94 (42.9)	125 (57.1)	3.556	1	0.069^{a}
≥ Tertiary level	141 (51.5)	133 (48.5)			
Monthly income					
<rm1000< th=""><th>51(39.8)</th><th>77(60.2)</th><th>12.024</th><th>3</th><th>0.007^{a*}</th></rm1000<>	51(39.8)	77(60.2)	12.024	3	0.007^{a*}
RM1001-1500	133(51.2)	127 (48.8)			
RM1501-2000	34 (41.5)	48(58.5)			
RM2001 and above	17(73.9)	6(26.1)			
Marital status					
Single	146 (49.8)	147(50.2)	1.353	1	0.245^{a}
Married	89 (44.5)	111(55.5)			

Number of children					
< 2	216 (48.9)	226 (51.1)	2.472	1	0.116^{a}
≥ 2	19 (37.3)	32(62.7)			
Duration of working					
experience in					
healthcare field					
3 - 6 months	38 (39.6)	58 (60.4)	8.429	2	0.015*
6-12 months	26 (37.1)	44 (62.9)			
≥12 months	171 (52.3)	156(47.7)			
History of attending					
training					
Yes	64 (74.4)	22 (25.6)	29.884	1	0.001*
No	171(42.0)	236 (58.0)			
Attitude					
Positive	153(71.5)	61(28.5)	86.067	1	0.001*
Negative	82(29.4)	197(70.6)			

^a Chi square test (X²)

3.4 Predictors towards good knowledge on maintaining vaccine cold chain

Table 4 illustrates the predictors towards good knowledge on maintaining vaccine cold chain. From multiple logistic analyses, there were three significant predictors found determining towards good knowledge on maintaining vaccine cold chain; monthly income (more than RM 2001 and above), positive attitude towards maintaining vaccine cold chain and with history of attending training related to the maintenance of vaccine cold chain. Based on Adjusted Odds Ratio (AOR) value, positive attitude was the strongest significant predictor towards good knowledge on maintaining vaccine cold chain. The odds of having good knowledge on maintaining vaccine cold chain among private healthcare assistants in Hulu Langat, Selangor with positive attitude are 5.8 more likely having good knowledge on maintaining vaccine cold chain compared to those with negative attitude. Meanwhile, the odds of having good knowledge among private healthcare assistants with monthly income more than RM 2001 and above are 4.5 times higher as compared to those having monthly income less than RM 1000. On the other hand, private healthcare assistants with history of attending training related to maintaining vaccine cold chain are 3.3 high likely to have good knowledge as compared to those without history of attending training.

Table 4: Predictors towards good knowledge on maintaining vaccine cold chain

Variable	β	SE	Wald	Adjuste d Odds	95% CI		<i>P</i> value
				Ratio	Lower	Upper	, 0.2020
Monthly income							
[<rm1000]< td=""><td></td><td></td><td></td><td>1</td><td></td><td></td><td></td></rm1000]<>				1			
RM1001-1500	0.440	0.260	2.868	1.553	0.933	2.584	0.090
RM1501-2000	0.041	0.345	0.014	1.042	0.530	2.049	0.906
RM2001 and above	1.504	0.592	6.455	4.499	1.410	14.353	0.011*

^b Fisher's Exact Test

^{*} Significant at P<0.05



History of attending training							
[No]				1			
Yes	1.177	0.302	15.195	3.246	1.796	5.868	0.001*
Attitude							
[Negative]				1			
Positive	1.761	0.212	68.984	5.821	3.841	8.8821	0.001*
Age							
[<26 years]				1			
\geq 26 years	-0.335	0.235	2.026	0.716	0.451	1.135	0.155
Number of children	Ĺ						
[<2]				1			
≥ 2	-0.787	0.385	4.175	0.455	0.214	0.968	0.051
Duration of working	g						
experience							
[< 12 months]				1			
\geq 12 months	0.275	0.240	1.309	1.316	0.822	2.108	0.253
Race							
[Malay]				1			
Non Malay	-0.240	0.249	0.931	0.787	0.483	1.281	0.335
Constant	-1.017	0.289	12.419	0.362			0.001*

4.0 Discussion

The maintenance of vaccine cold chain is important to ensure vaccines retain its efficacy in order to achieve the full benefit of immunization apart from high coverage and timely administered of vaccines. Healthcare staffs responsible for handling and administering vaccines play an important role to ensure vaccine cold chain is properly maintained. They must possess an adequate knowledge and skills pertaining to vaccine cold chain. This is important to avoid the possibility of vaccine cold chain failures as it is costly, time consuming and in many cases are avoidable.

This study found that majority of the respondents has poor knowledge on maintaining vaccine cold chain (52.3%). This is consistent with the fact that most of the respondents in this study have never attended training related to handling vaccine cold chain or information regarding vaccine cold chain was not taught by the employer or passed from senior colleagues who might have been trained previously. Similar finding was also reported from previous studies which highlighted inadequacy of knowledge on maintaining vaccine cold chain along healthcare workers (Yuan et al, 1995; Grasso, 1999; Naik et al, 2013; Oliveira et al, 2015). However, this finding contradicts with other studies conducted in Central Ethiopia (Rogie, Berhane & Bisrat, 2013) and Bandung (Mboe, Rahayuningsih, & Rusmil, 2012) where more than half of the respondents were rated to have satisfactory knowledge on vaccine cold chain. On the other hand, a huge difference can be seen in studies conducted at private health clinics Kelantan (Azira et al, 2013) and primary care unit in Turkey (Efe, A-Ncel & Ozer, 2008).) where a huge difference was seen in both studies with vast majority of the respondents have adequate knowledge of 78.7% and 96.3% respectively.





Three significant predictors were identified contributing towards good knowledge on maintaining vaccine cold chain which include; monthly income (more than RM2001 and above), history of attending training related to vaccine cold chain and positive attitude towards vaccine cold chain.

In this study, it was found that private healthcare workers with monthly income more than RM 2001 and above is 4.5 times more likely to have good knowledge on maintaining vaccine cold chain as compared to those with monthly income less than RM1000. Interestingly, higher monthly income was found to have a significant positive correlation with working experience in healthcare field (r=0.3, P<0.05). This explains that respondents with higher monthly income are more likely having longer working experience in healthcare field which in turn resulting to be more knowledgeable in maintaining vaccine cold chain. In addition, this can also be explained through the relationship between monthly income and educational attainment. As reported in a research done by Greenstone and Looney (2011), higher level of education affects lifetime salary with significant ability to boost earnings. He also explained that the monetary benefits of higher education can be seen in the lifetime difference of 65% earning power when comparing graduate salaries and the earnings of those with just high school education.

Second predictor that determines good knowledge on maintaining vaccine cold chain among private healthcare assistants was history of attending training related to maintaining vaccine cold chain. It was found that respondents with history of attending training related to maintaining vaccine cold chain are 3.3 times more likely to have good knowledge on vaccine cold chain as compared those without history of attending training. This finding is supported by several studies which revealed that training has significantly improve knowledge on vaccine cold chain maintenance among healthcare workers (Gopal-Krishnan, Sararaks, Amar-Singh, Amir & Ibrahim et al, 2014; Yakum, Ateudjieu, Walter & Watcho, 2015; Widsanugorn, Suwattana, Rashid & Sakamoto, 2011 and Bankole, Olusegun, Marian, Godswill, Adebowale & Lukeman, 2010). Basic knowledge on maintaining vaccine cold chain is important to ensure vaccine potency is secured and vaccine cold chain is not compromised. Unfortunately, trainings related to vaccine cold chain maintenance are not part of private primary care priorities. It is always assumed that vaccine cold chain system in private primary care centers can be sufficiently managed by General Practitioner and it is also not worth sending healthcare assistants who are only working as part time staff. Trainings related to the maintenance vaccine cold chain are mainly conducted by government health centers and pharmaceutical suppliers which are openly offered to both public and private healthcare workers. Module of this training include identifying types of vaccines that are sensitive to the effect of freeze and heat, monitoring and recording temperature of refrigerator, correct placement of refrigerator in the clinic and understanding the protocol of action needed during emergency cases. Main concern regarding attending training among private healthcare assistants is employee's opportunity of attending trainings that heavily depends on the employers own initiatives and awareness regarding the importance of vaccine cold chain.

Positive attitude was also found to be one of the predictors in determining good knowledge. Good perception on the importance of maintaining vaccine cold chain is directly linked with the knowledge on vaccine cold chain. In this study, it was found that respondents with positive attitude are 5.8 times more likely to have more knowledge as compare to respondents with negative attitude. Relationship between attitude and job satisfaction and performance among workers has been studied by Ghafoor and Maryam (2015) who found a significant



positive correlation between positive attitude and job performance and job satisfaction. The same study explained that an employee with positive attitude is more likely to have more job satisfaction and better performance which indirectly linked with better ability of gaining more knowledge at work through learning process at workplace.

On the other hand, five factors have been found to have significant associations with respondent's knowledge on vaccine cold chain from an inferential statistics. These factors include; sociodemographic characteristics (race and monthly income), history of attending training, duration of working experience in healthcare field and attitude towards maintaining vaccine cold chain.

In terms of race, proportion of Malay respondents with good knowledge was higher as compared to Non Malay respondents. A significant association was also found between highest level of education and race (χ^2 =29.366, df =3, P<0.0001), whereby Malay has the highest proportion of respondents with highest level of education up to college and above. This could explain higher number of Malay with good knowledge on maintaining vaccine cold chain. However, this finding was inconsistent with a previous study by Azira et al. (2013) on comparing the knowledge among healthcare worker on maintaining cold chain according to race difference which found that Non Malay respondents (85.7%) have more knowledge compare to Malay respondent (77.5%).

Meanwhile for monthly income, results showed that respondents with monthly income more than RM2001 and above have higher proportion of good knowledge as compared to other respondents with lower monthly income. Intuitively, higher salary could have a positive effect on job motivation and performance thus producing better result. Thus, respondents with higher monthly income are more motivated to gain more knowledge in order to produce better performance.

The association between respondent's knowledge of maintaining vaccine cold chain was also significantly associated with history of attending training related to vaccine cold chain (P<0.05). Majority of respondents with history of attending training related to vaccine cold chain have good knowledge on maintaining vaccine cold chain compared to respondents who have never attended any training. Training on maintaining vaccine cold chain covers all stages of storage and handling of vaccine cold chain system starting from where the vaccine was manufactured to the point it was administered. It does not only provide information on vaccine cold chain system but it also involves practical sand discussion session. This is important in exposing healthcare workers on handling vaccines cold chain system to be applied in real situation. A study done conducted in 4 regions in Gyeongsangbuk-province has also proven similar finding. Knowledge on maintenance of vaccine cold chain among vaccine administrators in private medical institutions was found to have significant improvement (P<0.001) after training has been given prior to that (Lee et al, 2012). This finding was also supported by several studies among healthcare workers done in private practice in Malaysia (Gopal-Krishnan et al, 2014), privately owned health facilities in Lagos, Nigeria (Bankole et al, 2010), hospital setting and health centers in Kalasin, Thailand (Widsanugorn et al, 2011) and health districts in North West Region of Cameroon (Yakum et al, 2015). These studies have clearly revealed that attending training related to maintaining vaccine cold chain is vital to ensure healthcare workers have adequate knowledge in handling vaccine cold chain system.



In general, knowledge is increased with experiences and expertise through knowledge sharing among colleague. The longer a person working in the field, the higher the opportunity of acquiring knowledge related to work field. This is reflected in this study where knowledge of maintaining vaccine cold chain was significantly associated with duration of working experience in healthcare field (P<0.05). It was found respondents with duration of working experience more than 12 months have high proportion of good knowledge of as compared to those respondents with 3-6 months and 6-12 months working experience in healthcare field. Similar finding was found in a study by Ademuyiwa et al (2014) which revealed that healthcare workers with more working experience of handling the vaccine cold chain in primary health clinics are more knowledgeable compared to those with less working experience ($X^2=33.8$, P<0.05). In addition, a study done in Kalasin, Thailand also revealed significant finding that healthcare workers working with more than 2 years of working experience have more knowledge compared to those with less working experience (P<0.001) (Widsanugorn et al, 2011). On the other hand, a different finding was reported in a study done in Turkey which reported that most of the healthcare workers with who have less working experience were more knowledgeable compare to those with more working experiences in healthcare field (Efe et al, 2008). Similar finding was also revealed in a study by Azira et al (2013) which reported that total working experience was significantly associated with knowledge and practice on vaccine cold chain (P<0.05).

Knowledge and attitude are two important interrelated factors affecting each other. People with positive attitude do not just possess the information regarding vaccine cold chain, but they are also able to appreciate the importance of maintaining vaccine cold chain. This study revealed that knowledge of maintaining vaccine cold chain among private healthcare workers was significantly associated with their attitude towards vaccine cold chain (P<0.05). Majority of respondents with positive attitude have higher proportion of good knowledge on maintaining vaccine cold chain. On the other hand, majority of respondents with negative attitude have higher proportion of poor knowledge. This finding was similar with a study in Bandung conducted among midwives managing vaccine cold chain in private healthcare practice which also revealed significant association between knowledge of vaccine cold chain and attitude towards vaccine cold chain (Mboe et al, 2012).

Overall, knowledge on maintaining vaccine cold chain among private healthcare assistants in Hulu Langat District, Selangor are regard as poor. Although majority of the respondents had longer working experience in healthcare field more than 12 months, yet the knowledge was poor. This could be explained by lack of training related to maintaining vaccine cold chain attended by vast majority of healthcare assistants which further lead to negative attitude towards vaccine cold chain system. Therefore, continuous training and surveillance on vaccine cold chain should be emphasized to improve knowledge and ensuring the effectiveness of immunisation programme.

5.0 Conclusion and recommendation

This study concluded that knowledge on maintaining vaccine cold chain among the majority of private healthcare assistants in Hulu Langat, Selangor was poor. Factors that were significantly associated with knowledge on maintaining vaccine cold chain among private healthcare assistants were race, monthly income, history of attending training related to



vaccine cold chain, duration of working experience in healthcare field and attitude towards vaccine cold chain maintenance. The significant predictors of good knowledge on maintaining vaccine cold chain were; monthly income (more than RM 2001 and above), history of attending training related to vaccine cold chain as well as attitude towards vaccine cold chain maintenance. Quality improvement activities such as continuous training and supervision as well as ensuring availability of educational materials may improve knowledge related to the maintenance of vaccine cold chain.

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Authors contribution

Author 1: information gathering, preparation and editing of manuscript

Author 2: editing of manuscript and final review of manuscript

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