CLEAN FRONTLINE

STATISTICAL ANALYSIS PLAN

Study title	CLEAN FRONTLINE CAMBODIA: A Stepped Wedge Cluster Trial of an Environmental Hygiene Educational Intervention across Thirteen Cambodian Hospitals
Short title	Clean Frontline
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1 Background

Environmental hygiene is a key component of infection prevention in healthcare, and a driver of healthcare associated infections. Staff who clean in many low resource countries receive no formal training on cleaning, waste disposal and linen handling. This issue has been exacerbated by the COVID-19 pandemic. The only recommended training on environmental hygiene for low resourced facilities, TEACH CLEAN, uses a training of trainers model. A selected cadre of "champions" is trained which in turn train their peers with responsibilities on environmental hygiene at the facility level. Early pilot data to test its effectiveness of this training package are very promising.

We will evaluate the effectiveness of an environmental cleaning bundle to improve microbiological cleanliness in Cambodian hospitals.

2 Objectives

The main aims of the trial are to assess the extent of 1) improvement in microbiological cleanliness of near-patient surfaces (primary outcome), and 2) decrease/reduction in the level of contamination and presence of an important healthcare pathogen, S. aureus.

3 Study Methods

3.1 Trial design

This study is a multicentre stepped wedge cluster randomized trial conducted in thirteen public hospitals (the trial clusters) in Cambodia. This study has four distinct steps. In each of the steps, either 3 or 4 hospitals (picked at random) will receive the intervention.

The intervention is based on the training package published in March 2023 by the World Health Organisation (WHO) entitled "Environmental cleaning and infection prevention and control in healthcare facilities in low- and middle-income countries".[20] The intervention relies on a pre-publication version of this package and its predecessor, TEACH CLEAN, on which the WHO package is primarily based. The intervention consists of three main phases. Phase 1 consists of preparatory activities, and it involves all hospitals at the same time. Phase 2 is the training of the Cleaning Champions, the timing of which is established by randomization. Phase 3 is the ongoing mentorship of cleaning champions while they trained and supervised cleaning staff. We consider the trial intervention subject to randomization as the training of facility cleaning champion (phase 2) and their mentorship (phase 3).

		2022								2023	
	Clusters/HOSPITALS	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DEC EMBER	FEBRUARY	MARCH
			PERIOD 1	*PERIOD 2*		*PERIOD 3*		*PERIOD 4*		*PERIOD 5*	
Sequence 1	1	0	0	1	1	1	1	1	1	1	1
Clusters = 3; Cluster size = 30	2	0	0	1	1	1	1	1	1	1	1
	3	0	0	1	1	1	1	1	1	1	1
Sequence 2	4	0	0	0	1	1	1	1	1	1	1
Clusters = 3; Cluster size = 30	5	0	0	0	1	1	1	1	1	1	1
	6	0	0	0	1	1	1	1	1	1	1
	7	0	0	0	0	0	1	1	1	1	1
Sequence 3	8	0	0	0	0	0	1	1	1	1	1
Clusters = 4; Cluster size = 30	9	0	0	0	0	0	1	1	1	1	1
	10	0	0	0	0	0	1	1	1	1	1
Sequence 4	11	0	0	0	0	0	0	0	1	1	1
Clusters = 3; Cluster size = 30	12	0	0	0	0	0	0	0	1	1	1
	13	0	0	0	0	0	0	0	1	1	1

Figure 1. Stepped wedge design with intervention (1) and control periods (0). The intervention period represents the time when the training occurred at the facility level.

3.2 Randomization

We used constrained randomization to ensure there was balance in two hospital characteristics in each randomization step: hospital level (i.e. Complimentary Package of Activities – CPA – which defines the type of clinical services offered: level 3 – the highest level- vs levels 1 or 2) and the region (i.e. within Battambang province vs other provinces). There were, in total, three CPA3 hospitals, one in each province, and seven hospitals situated in Battambang. We performed a public randomization – picking from "a hat" – in a virtual ceremony in February 2022, which involved all the key study coordinators (NIPH, WaterAid, DHS and LSHTM). Keeping the constrained rules described above, a representative from each coordinating organization had one of three hats (one for CPA3 hospitals; one for Battambang non-CPA3 hospitals; one for other provinces and non-CPA3 hospitals); each representative picked one cluster from their hat for each of the four designed steps. The procedures for randomization were agreed with an independent statistician.

3.3 Sample size

We calculated that with 30 surface samples in each hospital and with a pre-intervention cleanliness proportion ranging from 30% to 50%, we would have 85% power to detect a 30% relative increase in microbiological cleanliness. This is based on a two-sided 5 % significance level, a two-period decay correlation structure with conservative estimates of within-period intraclass correlation coefficient (ICC) in microbiological cleanliness at 0.03 (previous pilot), observation auto-correlation at 0.6 and cluster auto-correlation at 0.6. The pre-intervention cleanliness proportion and the within-hospital ICC were estimated using pilot data from similar-level maternity wards in Tanzania as no local data was available in Cambodia on microbiological cleanliness. For the power calculations, we used the website (https://clusterrcts.shinyapps.io/rshinyapp/) and underlying formula operationalized by Hemming et al. (2020) in March 2021.

3.4 Framework

All comparisons performed in this trial will be for superiority, with the hypothesis that the cleaning intervention will improve cleanliness compared to the control arm.

3.5 Statistical interim analysis and stopping guidance

There are no interim analyses planned and no stopping guidance.

3.6 Timing of final analysis

The final analysis will be performed after the last hospital site visit is conducted and the data have been cleaned. The plan is for the last visit to be undertaken in March 2023, with analysis in summer 2023.

3.7 Timing of outcome assessment

The primary outcome measure was a binary assessment of the cleanliness of hospital surfaces (binary clean versus not clean) performed using dipslides. We collected 30 double-sided dipslide samples from surfaces in three wards in each hospital every month: 12 from the maternity ward, 10 from the pediatric ward and 8 from a general medicine ward. Data collection was planned once a month for 10 months in each of the 13 hospitals. The time of the visit varied each month so that the hospital staff did not know when it would occur.

4 STATISTICAL PRINCIPLES

4.1 Confidence intervals and p-values

We will present 95% confidence intervals and p-values. However, we will not claim significance based on a p-value cut off point. In line with current debates, we will focus on the effect of the intervention, plausible mechanisms via which intervention would work, data quality, study design, bias and the real-world impact, rather than p-values in isolation.

4.2 Analysis population

There are three analysis populations:

1. an individual sample level population, consisting of every surface sample taken during the study,
2. a hospital-level population, consisting of hospital-level summaries of the outcome variables (averages for continuous variables, proportions for binary variables)
3. a ward-level population, consisting of ward-level summaries of the outcome variables (averages for continuous variables, proportions for binary variables)

5 TRIAL POPULATION

5.1 Eligibility

We approached all eligible hospitals [i.e. all public district or provincial referral hospitals]to maximise the feasibility of the trial; all participated. The hospitals are located across three selected provinces in Cambodia: Kampong Chhnang, Battambang and Kratie.

5.2 *Information to be included in the CONSORT flow diagram*

Reporting is based on the extension of the CONSORT guidelines for stepped wedge cluster randomised studies

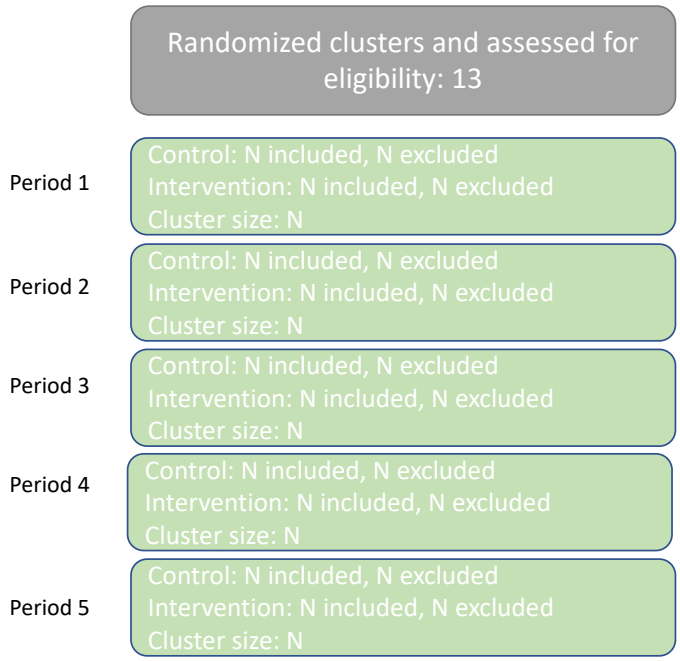

Figure 2 (Shell figure). Flow diagram

5.3 *Withdrawal / Follow-up*

In the event that a hospital withdraws from the study after randomization it will not be replaced, as we have already recruited all eligible hospitals.

5.4 *Baseline patient characteristics*

Baseline characteristics will be described by "randomisation step" and the following statistics will be reported: mean, standard deviation, median, IQR, for the following characteristics: cleanliness, temperature, patient volume and number of beds per cleaner.

Table 1 (Shell table)**.** Baseline characteristics (month 1 and 2) of the surface samples.

Sequence: Dipslides analyzed Estimate Number of hospitals	S1 % or mean (SD) 3	S2 % or mean (SD) 3	S3 % or mean (SD) 4	S4 % or mean (SD) 3

Primary binary outcome				
Cleanliness				
Pre-specified confounders				
Temperature (degrees)				
Patient volume (number of patients)				
Number of beds per cleaner				

6 ANALYSIS

6.1 Outcome definitions

6.1.1 Primary outcome

A surface will be rated as "clean" if it has an Aerobic Colony Count (ACC) less than 2.5 colony forming units (CFU)/cm^2.

6.1.2 Secondary outcomes

There are two secondary outcomes:

- the estimated number of CFU per slide
- presence or absence of S. aureus from the selective media on the other side of the dipslide

For the estimated number of CFU per slide we will use the categorical scoring of contamination (as shown below) and use the used the category mid-point in analyses as a continuous outcome. We reversed the order so higher number indicate greater cleanliness (100 – midscore). For example

Original category range	Contamination score used in analysis
0	100
>0 to <2.5	98.75
2.5-12	95.25
12-40	74
>40-	30

100	0

6.2 Analysis methods

For the primary outcome (binary: clean vs not clean) we will use a generalised estimating equation (Stata **`xtgeebcv`** command) with exchangeable equation structure using sample level data (i.e. surface sampled), accounting for within hospital clustering, with small sample Kauermann-Carroll correction. We will use a binomial distribution and logit link to estimate an odds ratio. Each cluster, hospital, will be analysed according to the actual time when training occurred independently of when training was planned

For all analyses we will adjust for the design factors: stratification variables which are CPA – hospital level - and province, both binary; and calendar time included as a categorical variable in the model). We will also adjust for four a priori potential confounders:

- number of beds per cleaner at the hospital level (linear),
- number of patients on the ward (linear)– time-dependent (monthly measurements)
- temperature of the ward (linear) – time-dependent (monthly measurements)
- surface type (3 categorical levels)

We will use a similar strategy for secondary outcomes. For 2.1 we will use generalized estimating equations again using `xtgeebcv` with a normal distribution and the identity link function.

We will in addition also conduct similar analyses on ward- and hospital-level averages for all outcomes. In these analyses, we will include a single variable which indicated the proportion of samples taken from bed rails (the largest category).

The main results will be presented in a table as follows:

Table 2 (Shell table). Unadjusted and adjusted primary and secondary outcome results estimating the effect of the intervention on microbiological cleanliness a) clean vs unclean; b) contamination score.

	Unadjusted*	**CI**	**Adjusted****	**CI**	**p-value**
Primary (pre-specified) outcome					
Individual samples dataset					
a) Binary clean vs no clean **(Unit: odds ratio**)					
Exploratory (pre-specified) outcomes					
Individual samples dataset					

b) Contamination score (Unit: number of CFU per displide)					
Hospital/Ward summaries dataset					
a) Binary clean vs no clean **(Unit: avg %)**					
b) Contamination score (Unit: average CFU per dipslide)					

*Accounts for clustering, time, and stratification variables

** Accounts for clustering, time, and stratification variables. Adjusted for temperature, patient volume, number of beds per cleaner and surface type.

6.3 Missing data

There is no plan to impute any missing data (neither outcome nor covariate data)

6.4 Additional analyses

We will estimate the absolute risk *difference* of binary outcomes, using generalized estimating equations with an identity link function, and a standard binomial distribution.

We will calculate the ICC for the primary outcome.

6.5 Harms

Health outcomes on individual patients are not measured in this trial. We will investigate unintended consequences using thematic analysis from the process evaluation data, but no formal statistical analysis will be performed.

6.6 Statistical software

Stata version 17 (or later) will be used for all analyses.