Study Title: A Multi-level Antimicrobial Surface Coating for a Healthier Environment

Protocol for the use of multilevel antimicrobial coating on patient privacy curtains in Kowloon Hospital

Approved by Clinical Research Ethics Review Committee, (Operation) (Kowloon Central/ Kowloon East)
Hospital Authority, Hong Kong

Project Approval Number: Approval Number: KC/KE-16-0189/ER-2
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Investigation Team:

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Site information: Kowloon Hospital (147A Argyle Street, Kowloon, Hong Kong)
Collaborators: 1. Hong Kong University of Science and technology, Hong Kong
2. Innovation and Technology Fund (ITF), Hong Kong

Preparation site: Core lab (Pathology Unit), 3rd floor, main building, Kowloon Hospital
Sampling Site: Rehabilitation ward 3B,

- Male cubicle (3rd cubicle)
    - No of beds: 8
    - No curtains: 12
    - Available Nursing counter: yes (13 staff members including nurses and supporting staff)
    - Visitors allowed: Yes, (12:00-13:00 and 17:00-19:00 every day)
    - Additional cubicle for 3rd phase of study: 2nd cubicle, 4 beds, 4 curtains
- Female cubicle (3rd cubicle)
    - No of beds: 8
    - No curtains: 12
    - Available Nursing counter and Head nurse office: yes (15 staff members including nurses and supporting staff)
    - Visitors allowed: Yes, (12:00-13:00 and 17:00-19:00 every day)
    - Additional cubicle for 3rd phase of study: 2nd cubicle, 4 beds, 4 curtains

Material Requirement:

1. Antimicrobial coating (C16): 100 Liters/ every 3rd week (for each phase)
2. Prepare duplicate Agar plates of
    a. Chromagar MRSA: Selective media for MRSA (ca. 4000 plates)
    b. TSA (Tryptic soya agar): for total bacteria count (ca. 4000 plates)
3. Prepare of neutralizing solution: 20 liters
4. Preparation of Polywipe sponge: 2000 (40 boxes)
5. Preparation of buffer solution for dilution (5 liters)
6. Labelling of Agar plates (ca. 8000 plates)
7. Drying of Agar plates at room temperature
8. Autoclave for Sterilization after enumeration

Study Status: (Completed)

Summary

- Submission of study protocol to ethical review committee: June 2017
- Approval of clinical trials by Research Ethics Committee (Kowloon Central/Kowloon East): Aug 2017

- Completion of background/pilot study: Oct 2017
- Completion of data collection in three phases: June 2018

Sampling Duration, Time Frame, and work plan:

Background /Pilot Study	**Survey**	**Observation Period (1st Data Set/Phase 1)**	**Washout**	**Survey**	**Observation Period (2nd Data Set/ Phase 2)**	**Washout**	**Survey**	**Observation Period (3rd Data Set/ Phase 3)**
8 weeks	**4 weeks**	**4 weeks**	**3 week**	**2 Week**	**4 weeks**	**3 week**	**2 week**	**4 weeks**
04 Sep -30 Oct (2017)	**31 Oct – 28 Nov (2017)**	**01 Dec - 30 Dec (2017)**	**1st Jan - 05 Feb (2018)**		**06 Feb – 08 Mar (2018)**	**26 Mar – 30 Apr (2018)**		**22 May – 20 Jun (2018)**
		8 curtains from Male **12 curtains from Female Cubicle** **50% coated curtain, 50% standard curtain**			**12 Curtains from Male Cubicles** **12 Curtains from Female Cubicle** **50% coated curtain, 50% standard curtain**			**16 Curtains from Male Cubicles** **16 Curtains from Female Cubicle** **50% coated curtain, 50% standard curtain**
		No of samples: 480			**No of samples: 576**			**No of samples: 768**

Team Members:

HKUST

1. Prof King Lun Yeung (Principal Investigator)
2. Prof Joseph Kwan (Co-Investigator)
3. Dr. Farid Awais (PhD Student)
4. Dr. Chang Qing (Post Doc)
5. Mr. Bryant (MSc Student)
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8. Mr. Robben (Research Assistant)
9. Ms. Song xizi (Research Assistant)

Kowloon Hospital

1. Dr. Dominic Tsang (Co-Investigator)
2. Dr. Christopher Lai (Co-Investigator)
3. Ms. Yeung Kin Yuk (ICT Nurse)
4. Mr. Kennedy Tse (ICT Nurse)
5. Ms Mary Tse (Core-lab In-charge)
6. Ms Kari Hung (Core-lab In-charge)
7. Ms Mo Mei Mei (Ward Manager)
8. Mr. H K Tsui (Media Preparation)

Brief Summary of Project:

This project aimed to study the use of the multi-level antimicrobial coating in a working hospital environment. Patient privacy curtains from a public sector hospital were coated and installed in rehabilitation ward in comparison of normally washed curtains in the same setting and compared the mean reduction on both control and treatment end to assess the effectiveness of coating against hospital acquired infections including MDROs.

Detailed Description:

[1] A multi-level antimicrobial coating was produced in the HKUST laboratory using the newly developed staged flow micromixing to prepare contact-killing and anti-adhesion coating made of US-FDA approved polymeric materials. The process was optimized to scale-up the production to 5 liters per hour. 300 liters of the multi-level antimicrobial coating was prepared for the study for a total of 24 weeks as three liters of the coating was needed for one patient privacy curtain.

[2] The study was conducted in the rehabilitation ward of the Kowloon Hospital including both male and female cubicles. It was carried out in three stages with each stage consisting of survey and a four weeks' observation period, separated by three weeks' washout period. In the rehabilitation ward of Kowloon Hospital, a set of male and female cubicle was recruited for the study after getting maximum number of bacterial contamination on patient privacy curtains in pilot study of 8 weeks. Each cubicle had 12 curtains besides admitted patients where all the samples are collected.

[3] Environmental sampling was carried out using sponge swab on the surfaces of the patient privacy curtains. The total bacteria count and quantitative isolation of MRSA was done using established protocols. The total bacteria count provides a quantitative measure of surface cleanliness, while the MRSA count was indicative of the risk of contact transmission from contaminated surfaces. 2-4 weeks survey provided control data on the cleanliness of patients privacy curtains in terms of total bacteria and MRSA counts.

During the study period, the Kowloon Hospital/Queen Elizabeth Hospital infection control team monitored MDROs regularly as part of their routine operation. The procedure and schedule was followed strictly the infection control protocol. All infection control measures implemented after isolation of MDROs were followed according to the hospital's usual practice as advised by the infection control team. In the study, the identification of the organisms in patients more than 48 hours after admission without prior isolation of the organisms in clinical or screening specimens was defined as nosocomial.

[4] The investigators demonstrated a cross-over intervention study. In the first stage of study, half of the patient privacy curtains were considered as treatment (antimicrobial coated) and the other

half of the curtains as control in the same setting in a cubicle. As the study was double blind, so coding was done to identify the treatment as control curtains.

[5] Sample were taken from eight highly touched areas of 50x50 cm^2 on weekly bases for three consecutive weeks on 7th day of first installation. On each period/phase 480, 576 and 786 samples were collected accordingly using the developed sample protocol. In total, the investigators collected 1824 samples from 76 patient privacy curtains in which 912 (50%) were control curtain and 912 (50%) are antimicrobial coated curtains.

[6] Healthcare workers from the participating wards were assessed for their acceptance of the multilevel antimicrobial disinfectant coating by way of a questionnaire. Additional reformulation may be necessary to increase the acceptability of the coating technology to the healthcare workers.

[7] For the bacteria count, duplicate plates of TSA (total bacteria count) and Chromagar MRSA (selective agar for MRSA detection) were used for enumeration. CFU/m^2 is calculated after 48 hours' incubation at 37C. Data was analyzed using SPSS V.21 to assess the effectiveness of the coating in reducing the contamination level of the surface. The total bacteria count on the patient privacy curtains was compared between the control and treatment group using Mann-Whitney test and T-test. ANOVA analysis allowed the comparison of different periods of study of phase wise as well as week wise. The significance of the statistical test is defined to have a P value <0.05.

Study Design:

Interventional cross-over study

Study Objectives

1) To compare the antibacterial effect of in use antimicrobial coated patient privacy curtains with standard curtains:

i) Total bacterial count over time
ii) Time of first MDRO contamination
iii) Percentage of MDRO contamination

2) To assess healthcare worker's acceptance of the multi-level antimicrobial coating.

Inclusion Criteria

For the clinical setting of the allocated ward with a prior history of high MDRO (preferably MRSA) prevalence is included.

Exclusion Criteria

For the clinical setting of the allocated ward with a prior history of low MDRO prevalence is excluded.

Primary Outcome Measure:

1. Change in total bacteria count and MRSA in control vs treatment patient privacy curtains

To quantify the effectiveness of antimicrobial coating, percentage change (both in log and linear scale) in mean bacterial count (CFU/m2 units) in control versus treatment curtains is used. The data was collected in 3 phases of 4 weeks each, so the time frame used is representative of the complete observation period.
[Time Frame: 12 weeks]

2. Durability of antimicrobial coating in affecting bacterial load amongst treated patient privacy curtains

Change in bacterial load amongst treatment curtains is observed as a function of time during each phase of the data collection period (for 4 weeks). In total, data was collected in 3 phases of 4 weeks each, so the total observation period is 12 weeks and timeframe of each frame is 4 weeks.
3) [Time Frame: 12 weeks]

Secondary Outcome Measures:

1. Agreement/disagreement of hospital staff regarding technology acceptance and adaptation

A survey was conducted using a self-structured questionnaire to get feedback from the hospital staff regarding their acceptance of the technology. The questionnaire contained questions regarding the physical (smell, appearance and feel) aspects of the coating as well as general approval/disapproval based on their experience with the technology. A scale of -5(worst) to +5(best), with 0 representing neutral was used, representing a dimensionless quantity based on staff's personal preferences. Mean of the user's response to each question was used for quantification. The users observed the technology during the entire 12 weeks of the study period (during all 3 phases of data collection). Users were give a 4-week time period to submit their survey responses.
[Time Frame: 4weeks]

Arms	Assigned Interventions
Active Comparator: Regular Patient privacy curtain Control arm is a regular patient privacy curtain, washed and dried in hospital laundry using commercially available sodium hypochlorite and hydrogen peroxide.	**Combination Product**: Regular Patient privacy curtain Laundered curtains, used in the hospital, coming from the hospital inventory.
Experimental Comparator: Antimicrobial Coated Curtains Treatment arm is antimicrobial coated curtains that are coated by dipping method, dried and provided to nursing/supporting staff to change after every 3-4 weeks.	**Combination Product**: Regular patient privacy curtains Antimicrobial Coated curtains

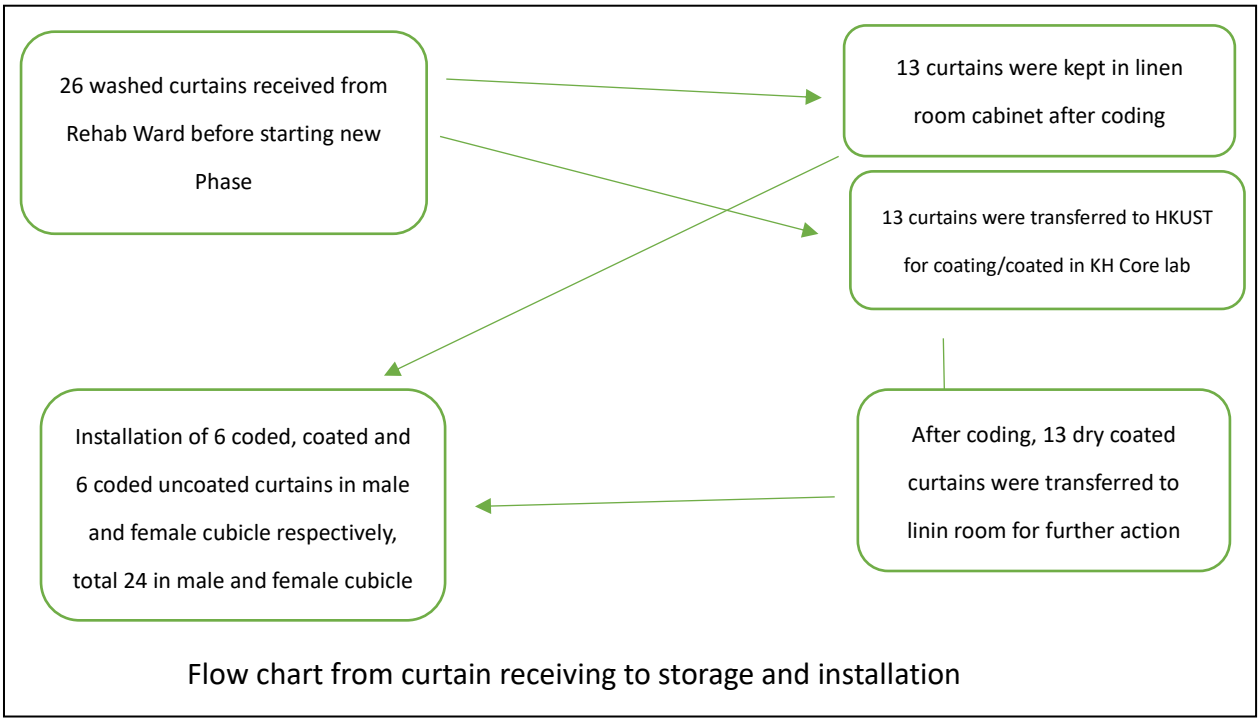
Protocol:

1. Curtain receiving, coating, coding, and transportation:

1. Every 2nd week, HKUST team received 26 washed (routinely cleaned) curtains from rehabilitation ward linin room on ground floor.
2. 13 curtains were kept in a designated cabinet in the linin room at 3rd floor Rehabilitation ward 3B. These 13 curtains were coded according to assigned numbers as control curtains.
3. 13 remaining washed curtains out of 26 were transported to HKUST by using three large luggage bags
4. As each curtain absorbs approx. 3 Liters of the coating solution, so our team had ensured availability of 30 liters of C16 coating material on completion of each phase.
5. Our team preferably prepared fresh C6 coating and diluted C6 in tab water at the time of coating to prepare C16.
6. We had three 95 Liters capacity big containers, that were used to coat the curtains in HKUST premises during Pilot study.
7. The best practice to coat the curtain was dipping method (dip a dry curtain in a filled container of C16 coating).
8. Curtains were soaked/dipped for 30 minutes in coating material to absorb properly and uniformly.
9. After soaking, curtains were squeezed gently to remove extra solution/liquid and transferred to clean and dry container.
10. 13 wet coated curtains were transferred to laundry to dry as following:
    a. Hot air of 65°C for 30 minutes.

b. Cold air of 20°C for 3-5 minutes to reduce the temperature of curtains.

11. 13 dry, coated curtains were brought back to the linen room of rehabilitation ward, coding was done according to the assigned number to keep the study blind. Only principal investigator has the knowledge of the factitious codes to keep study double blind.
12. All the curtains (13 coated and 13 uncoated) were prepared for installation in 1 week including receiving, transportation, coating, drying, and coding during pilot study.
13. <u>Drying protocol amendment</u>
    a. Drying protocol was amended due to shedding of coating in heavy circular mechanical motion in laundry dryer. For this purpose, curtains were kept on room temperature for overnight, and next morning when there was no apparent dipping, they were shifted to walk in incubator of Pathology lab at 50°C for 24-36 hours for best drying results.
    b. Drying in HKUST premises was difficult with minimum resources, heavy weight and humidity issue along with transportation.



Flow chart from curtain receiving to storage and installation

*1 extra pair of curtains for control and treatment end was kept as a back up

Fig 1. Illustration of coating in container, method of coating by dipping method, squeezing, transportation and drying of patient privacy curtains

2. Curtain installation:

1. Every 3rd week, our team changed the curtains from 2 cubicles (1 male and one female) according to curtain changing policy of hospital.
2. At the start of every phase of study, following the standard protocol; after taking official consent from ward manager we changed the curtains at once from both cubicles.
3. There were two types of curtains clippings, with metal ring and cloth strips so that curtains were changed according to the type of clips/ring.
4. As there was the capacity of 12 curtains in one cubicle, so six curtains were coated and six uncoated. The sequence was alternative as one coated next uncoated and so on. After installation, we informed ward manager about completion of curtain hanging in the designated ward and took first sample at day 7 because we gave sufficient time to curtains to be contaminate in hospital environment.
5. There was a backup pair of the curtain in case of need to change curtain due to spoilage, vomitus, blood or MDRO diagnosed the patient. Staff was informed to notify our team about the date and reason of curtain changing.

Fig 2. Illustration of curtains installation in cubicles as one control other treatment showing matched paired randomization

Environmental Sampling:

1. Sampling Frequency: Curtains were sampled on a weekly basis for the total duration of 3 weeks. So, every curtain was sampled three times during complete sampling duration. First 7 days was the time of exposure to contaminate leading with next 3 weeks of sampling.
2. Sampling Area: Each curtain was sampled for eight areas for the size of 50x50cm² each. Four areas from top side starting from patient's head side to toe side on the bed, and four areas from bottom side moving from toe to head side. As the size of curtain varies from 430cm to 480cm in length, for the best results after pilot study, we calibrated 8 areas keeping 50 cm from above, 37cm from lateral margins and 57-70cm in between.

- Area 1: Upper Head side
- Area 2: Upper 2nd area
- Area 3: Upper 3rd area
- Area 4: Upper Toe Area

- Area 5: Lower Toe Side
- Area 6: Lower 2nd area
- Area 7: Lower 3rd area
- Area 8: Lower Head Area

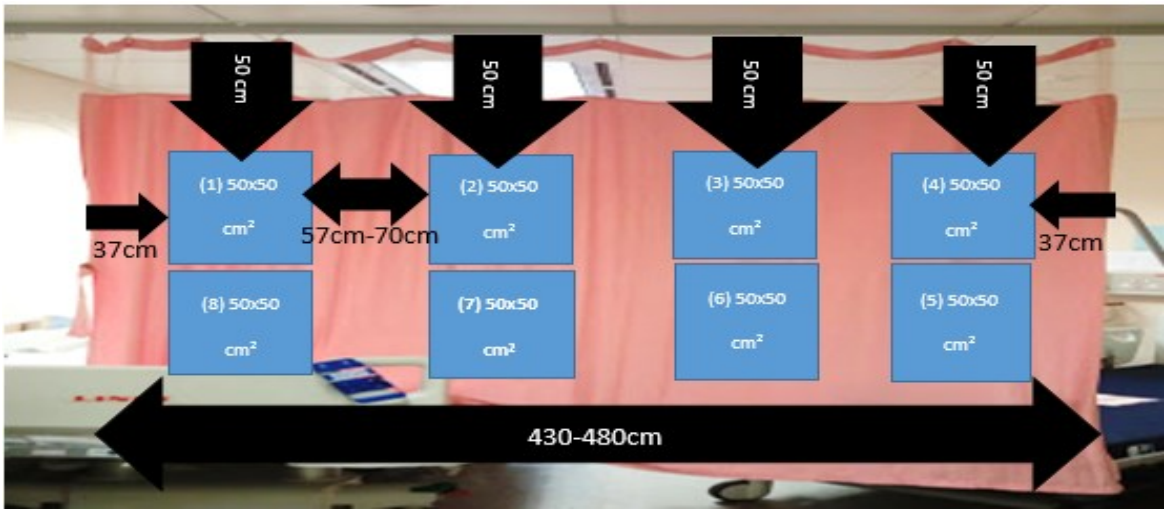


Fig 3. Illustration of 8 sampling location on a curtain received from Rehabilitation ward of KH, Designated 50x50cm² area were sampled following the protocol

3. Sampling Side of the curtain: Sampling was done from the inner side of the curtain, (inner side was more exposed to the risk of contamination as a frequent touch of the patient on the bed, nursing staff, cleaning staff, monitoring staff, attendants, and doctor).
4. Sampling material: Polywipe sponge were used to sample 50x50cm² area on the curtain.
5. Sampling technique: Both sides of a sponge were used by swabbing on the curtain. Holding the sponge from the middle of one side with the right hand, keeping vertically, swab area starting from upper left margin towards the upper right margin of 50x50cm² area from one side of the sponge. The same method was used swabbing from right to left side with other side of the sponge. Sampling was done gently, slowly and softly by not repeating the swab to sampled area.
6. Sampling frame: 50x50cm² frame of plastic covered with a disposable plastic sheet was used as back support for smooth sampling and accurate measurement. Every time, sterilized plastic was removed and new sheet was used to reduce the chance of microbial spread or false positive result on control and treatment arm of the study

Sample size calculation using G-power 3.1.9.2

In our previous study carried out in orthopedics wards, a total of 2249 samples were taken from hospital surfaces over a study period of 1 year. The following was the mean and standard deviation of log total-bacteria-count in the previous study.

Group Statistics

Type (1/2)		N	Mean	Std. Deviation	Std. Error Mean
log (CFU/m²)	treatment	1130	4.13126	.544325	.016193
	control	1119	4.32627	.531846	.015899

Basic information of previous field study in orthopedic ward

Assumptions:

1. The sample taken may have a higher standard deviation than the previous study. SD assumed to be 1log.
2. 0.21 log reduction on the total bacteria count on the bed partition curtains was assumed. This was based on the difference of the control and the treatment group in the previous study.

t tests - Means: Difference between two independent means (two groups)

Analysis: A priori: Compute required sample size

Input: Tail(s) = One

Effect size d = 0.2000000

α err prob = 0.05

	Power (1-β err prob)	=	0.95
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	3.2924155
	Critical t	=	1.6462631
	Df	=	1082
	Sample size group 1	=	542
	Sample size group 2	=	542
	Total sample size	=	1084
	Actual power	=	0.9500669

Hence a minimum of 1084 specimens were adequate for our study. To best fit the wards size and sampling schedule, we reckon a sample size of 1800 was best suited for our study.

Safety Training for investigators:

Infection control team of Kowloon Hospital provided safety training to all participants of research team. Infection control nurse described the safety measures according to hospital safety guidelines. After full day training, participants were assessed by written question about using of person protective equipment including mask, gloves, gowns, and caps. All successful participants were allowed to become a part of investigating team. Ward managers in pathology and rehabilitation wards also explained about emergency exits and dealing with emergency in case of any accident during working hours. Finally, all participants were provided with staff access cards with validity till completion of study (June 30, 2018).

Pre-sampling arrangement:

1. Preparation of neutralizing solution:
   a. Neutralizing solution: 1-liter preparation, (Polysorbate/Tween 80, 30 g/L; Saponin, 30 g/L; and lecithin, 3 g/L; pH 7.0).
   b. As normal preparation, pH was 5-6, so we used NaOH as a base to bring pH at 7.
   c. According to sampling plan the required 20 liters neutralizing solution was prepared on weekly basis by keeping it sterilized to prevent contamination that might gave false positive results.
2. Preparation of spreading beads:
   a. For 100µl solution spread on agar medial on agar plates, we poured 5-10 beads on each plate and shake gently until all liquid media spread and absorbed.
   b. All the beads were pre autoclaved and sterilized to prevent contamination.
3. Pouring of neutralizer into sponge containing zip bags:
   a. 10ml of neutralizing solution was poured into sterilized empty zip plastic bags.
   b. We used a new 10ml pipette glass tube and electric pipette to pour neutralizer on

operation bench in KH core lab, before sampling.

c. The team dealing with neutralizer pouring was operating carefully to measure the appropriate amount, prevent spoilage and maintain hygiene and safety.

d. Zipped bags were opened minimally, to reduce the chances of contamination.

4. Labelling:
   a. All the bags were labelled before sampling
   b. Labelled the bags with bed number, coding, sampling area
5. Transportation Containers:
   a. Sampling material containers were have following items to bring in rehabilitation ward (1 set for male and similar another set for female cubicle) for 1-time sampling:
      - Polywipe sponges 110, (required 96, extra 14 in case of contamination/ fall in floor during sampling)
      - Plastic gloves (blue colored), already given in polywipe sponge box
      - Plastic holing clips, to identify measured area
      - Measuring tapes, to measure 50x50cm² area as a sampling area
      - Neutralizing solution in zipped labelled bags 110, (required 96, extra 14 in case of contamination/ fall in floor/ leakage)
      - Autoclave medium size bag to put used gloves used sponge and wrappers of sponge

Sampling Arrangements and methods:

1. Sampling Cubicles (Location): one male cubicle (3rd cubicle) and one female cubicle were allocated for this study after pilot study results. These were located on 3rd floor or rehabilitation building (ward 3B).
2. Sampling time: Every visit, sampling was started at 8:30 am and finished till 12:00 pm.
3. Following the Rules: Sampling team had to follow the rules of Kowloon Hospital in rehab wards. The team had to sample curtains smoothly and quietly to minimize disturbance to working staff as well as patients in beds. The team was not allowed to encroach patient treatment or investigations/ file to keep the confidentiality.
4. Gowning for safety: All the members were gowned properly by wearing personal protective equipment (disposable gown, gloves, mask and head cover). For gowning, individual followed the step mentioned in safety training, as all sampling taking persons had gone through safety training by infection control team of Kowloon Hospital.
5. Sample taking notification and Consent: Team was notifying ward manager/ ward in-charge about sampling plan and take verbal permission to proceed.
6. Sampling Teams: In the female cubicle, two female team members took samples. One

member was take a sample of swabbing the area; other was helping to provide gloves, sponge, identification of appropriate area, calibrate the size and provide support by holding the frame of 50x50cm². The same sampling protocol was adopted in a male cubicle where male team members were taking the samples.

7. Safety precaution:

   Following safety measures were accomplished before and during sampling:

   a. Before sampling, clean the hands having gloves with available disinfectant.
   b. Put plastic disposable gloves and take out sponge from its plastic packing.
   c. Again disinfect plastic gloves and let it dry for few second.
   d. Hold the sponge firmly from one side and keep sampling designed 50x50cm² area.
   e. Don't touch any other, other area holding the sponge, and prevent felling down on contaminated floor
   f. In case, sponge was touch with other surface or fell on floor, please discard that sponge and resample the same area with a new sponge

8. Sample preservation:

   a. After proper sampling, we put the sponge in zipped plastic bag containing 10 ml neutralizing solution quickly to reduce chance of contamination
   b. 2nd team member opened the zipped bag for sampling member for convenient proceeding
   c. The 2nd team member zipped up the sampling bag aseptically, confirmed the correct labeling, and kept in an aseptic container.
   d. 2nd team member also mixed the neutralizer gently with a sampled sponge
   e. Sponge sample in neutralizing solution bag were kept in a safe container until all the sampling was not completed.

9. Degowning and Transportation:

   a. After completion of all samples from patient privacy curtains, the team were degowning according to the protocol by removing PPE.
   b. Transportation boxes were brought back to pathology core lab of KH from rehabilitation ward immediately after completion of sampling.
   c. Transportation containers were brought back by holding with one hand gloved another hand without gloves according to infection prevention policy

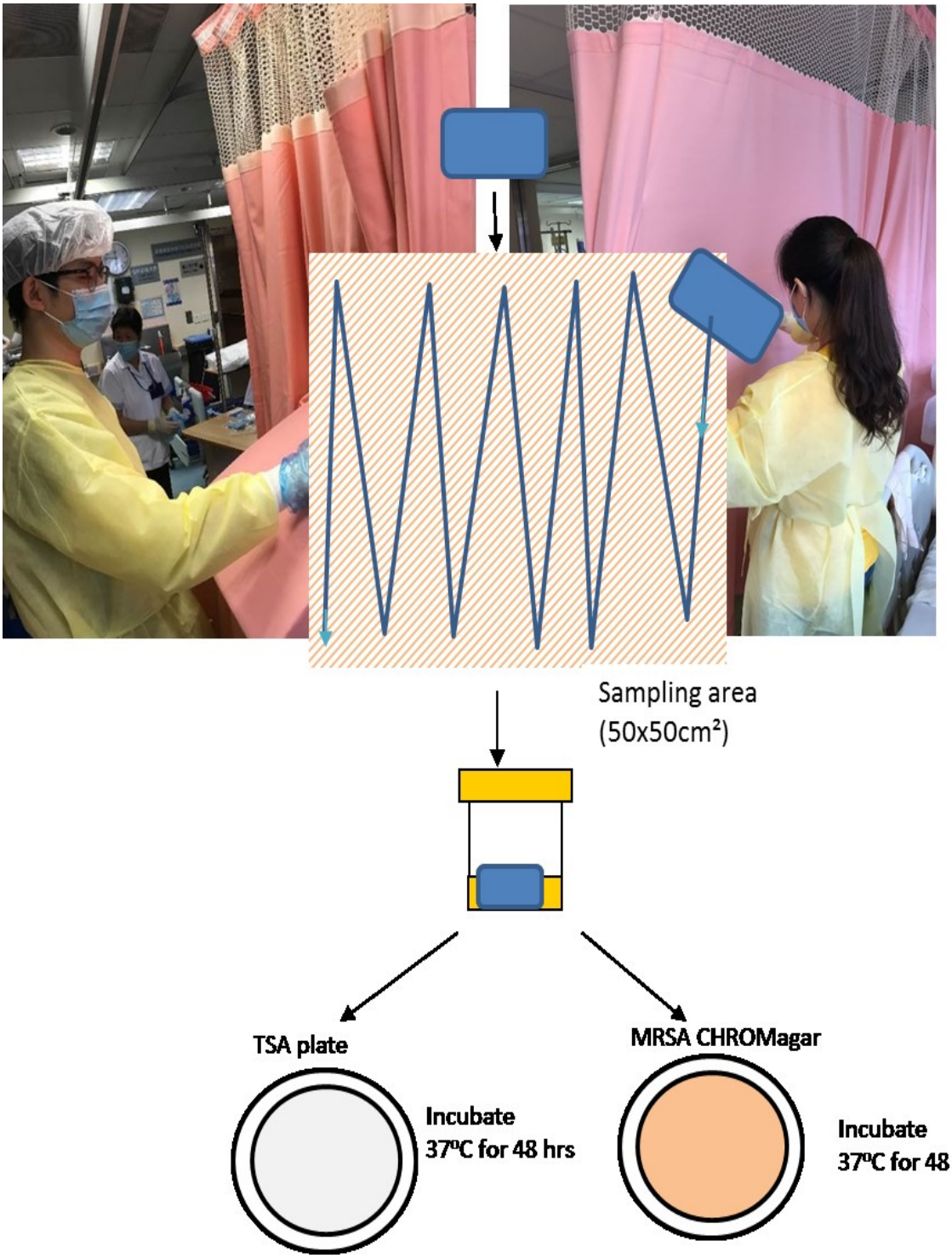
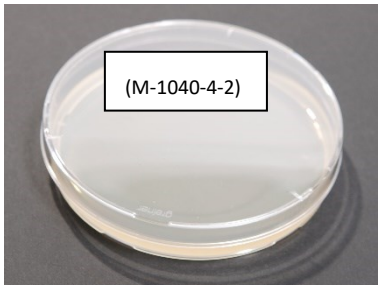


Fig 4. Illustration of sampling method on curtains in Kowloon Hospital, preservation and inoculation on TSA and Chromagar plates

Post Sampling Procedures:

1. Arrangements of Agar Media Plates: Queen Elizabeth Hospital's (QEH) Media preparation unit prepared TSA and Chromagar MRSA agar plates according to needs of this research.
    a. Principal investigator was notifying Mr. Tsui (coordinator for media preparation on QEH) two weeks ago about the preparation of required media plates.
    b. All the prepared media was preserved at 3-4^{o}C in QEH. Media plates boxes were labelled as HKUST-TSA and HKUST-MRSA plates in different batches.
    c. Prepared boxed were transferred to Kowloon Hospital's cold room for long term use as well as prevention from contamination.
    d. For the media preparation, sampling team coordinated by emails or meet them in person if there was any change in requirement.
2. Agar plates Standardization: As the agar plates were kept at lower temperature (3-4^{o}C), agar plates should normalize to normal room temperature as recommended. We kept out the agar plates on the desk before going for sampling, and in the meanwhile, plates were ready to use after 3-4 hrs.
3. Unboxing and labelling of agar plates: All the prepared plates were packed in boxes, so before using the plates, team was unboxing, calculating and separating two type of media plates. Each plate was labelled as
    a. Code, i.e., 1040
    b. Sampling area number (1-8)
    c. Plate number (first or duplicate)
    d. Location (Male/ Female)
        i.e. (M-1040-4-2)

    Male-code 1040- Area 4- sample 2



4. Sample inoculation area: Samples were inoculated on the desks in Kowloon hospital pathology core lab. All the team members were participating in this procedure following safety measure, wearing PPE.
5. Mixing of Sponges containing neutralizer bag: Before taking 100µl of a neutralizing solution, it was compulsory to mix sponge very well so that all suspected microbes would come out from the sponge into the solution. Mixing was gentle, continuous and for the period of a minimum 1 minute.
6. Dilution in PBS: 100µl of neutralizing solution was diluted in 900µl of PBS, mixed well and then100µl was put on agar plated to inoculate. This was performed for TSA plates because bacterial load was high and plates were unable to read.
7. Inoculation Procedure:
    a. 100µl of well mixed neutralizing solution was dropped by using 100 -200 µl pipette with sterilized pipette tube on Chromagar MRSA plates and spread homogenously

by sterilized beads by gentle shaking

b. 100µl of solution from PBS mixed solution was dropped by using 100 -200 µl pipette with sterilized pipette tube on TSA plates and spread homogenously by sterilized beads by gentle shaking
c. All the plated were shaked well in clock wise circular motion for 1-2 minutes for homogenous spread
d. When there was not a visible solution on agar plates, opened the agar plates and took out beads in autoclave bag respectively.
e. All inoculated agar plates were finally transferred to an incubator and kept for 48hours at 37^{o}C.
f. Inoculated plates were transferred in a specified labelled container to prevent any infection spread or spoilage

Fig 5. Illustration of incubation, enumeration and decontamination with autoclave in pathology ward of Kowloon Hospital

8. <u>Cleaning inoculation area:</u> All the desks were cleaned with bleach (NaClO) or other available disinfectant, recommended by hospital infection control authority using cotton wipes. All touched surface, including inoculated transfer container, was cleaned with recommended disinfectant.
9. <u>Results:</u>
    a. At 48hrs, all the plates were counted to calculate CFU/m^2.
    b. TSA (Tryptic Soya Agar) was used to count total bacteria. The colonies were pale to yellow in the circular pattern. The average number of colonies was 50-250 on TSA plates as PBS diluted factor.
    c. Chromagar MRSA was used a selective media to check MRSA. MRSA appeared as Purple to Pink rounded colonies. In case of yellow or blue colonies growth on media, we did not count those unknown gram positive bacteria.
    d. Counted plated were wrapped with plastic tape and put in autoclave bags to autoclave clinical waste. KH staff helped to autoclave used plates for safety measures.

e. After discarding counted plates safely, the counting area, material touched or exposed was cleaned using standard disinfection.
f. Results were saved in an official register for keeping the data in privacy
g. All the data was uploaded to excel sheet and analyzed for a bacterial reduction on comparative ends of control and treatment curtains.
h. Weekly, data was assessed and shared with Prof King Lung Yeung along with a central continuous excel sheet with raw data.
i. HKUST team also shared the results with KH and QEH research team on a monthly basis as a summarized report and brief power point briefing session in the conference room of KH after coordination.

Data Analysis:

The duplicate system in total bacteria count helped to monitor the validity of the data point. Logical elimination of plate count was determined while comparing the bacteria morphology of the first plate with the second plate. If the bacteria morphology was not consistent, the plate with more bacteria was eliminated, and the plate with a lower value was taken due to the chance of contamination on a non-selective agar plate.

The Colony Forming Unit (CFU) obtained on the duplicated plates was averaged, and the CFU per unit area was calculated. CFU per unit area was allowing the comparison of microbiological contamination of the sampled surface. With the dilution factor of the samples, the Total bacteria count (TBC) was determined. The microbiological contamination level of an item was represented by the CFU/unit area of the item.

CFU on Müller-Hinton Agar represents the total aerobic bacteria count. By dividing the CFU with the area sampled, CFU/m^2 was obtained. Data points that did not contain any CFU count were assumed to have 0.5 CFU. The assumption was made based on the minimum detection value that can be obtained.

All analysis was carried out using Statistical Package for the Social Sciences for Windows, version 25.0 (SPSS, Inc., Chicago, IL, USA) to analyze the effectiveness of the coating in reducing the contamination level of the surface. The total bacteria count on the patient partition curtains was compared to the control and treatment group using Mann-Whitley test and T-test. Chi-square test and Fisher's exact test were also used to compare categorical variables such as the percentage of MRSA. Parametric test was carried out to evaluate the cleanliness baseline in each of the wards before and after treatment. ANOVA analysis allowed the comparison of different periods of study and the differences among wards. Median time to first contamination (days) of MDRO for each curtain type was compared using the Wilcoxon rank-sum test. The significance of the statistical test was defined to have a P value <0.05.

Healthcare workers from the participating wards were assessed for their acceptance of the multilevel antimicrobial disinfectant coating by way of a questionnaire. Additional reformulation may be necessary to increase the acceptability of the coating technology to the healthcare workers.

Ethical Concerns:

The study involved an evidence-based and data guided intervention that was designed to bring about prompt improvement in the quality and effectiveness of healthcare deliveries in hospitals. The patient data collected by ICT did not involve identifiable personal data; and was regarded as part of standard care hence no further consent seeking was deemed necessary. The publish of study results is on the basis of the principle of justice since the study findings could direct limited resources pertaining to infection control and help to further reduce cross-transmission of infections in hospital setting.

Sampling and intervention were solely performed on the curtains and the care of the patient is not affected. All the laboratory forms and data files did not contain personal information, and only aggregated data was used in the final report or publications.