University College London Hospitals NHS
NHS Foundation Trust

Environmental Research Laboratory
University College Hospital
Room E -2PT001
EGA Wing
Level -2
235 Euston Road
London
NW1 2BU

Telephone: 020 344 76700
Email: shanom.ali@uclh.nhs.uk

DRAFT

Evaluation of the persistence of MRSA, *E. coli* and *K. pneumoniae* contamination on Sealwise WCB® anti-bacterial and standard Sealwise WCB® surfaces

Prepared for:

Sealwise Ltd
Unit 5 Mendip Business Park
Mendip Road
Axbridge
Somerset
BS26 2UG

www.sealwise.co.uk

February 2014

University College London Hospitals NHS
NHS Foundation Trust

Environmental Research Laboratory
University College Hospital
Room E -2PT001
EGA Wing
Level -2
235 Euston Road
London
NW1 2BU

Telephone: 020 344 76700
Email: shanom.ali@uclh.nhs.uk

Evaluation of the persistence of MRSA, *E. coli* and *K. pneumoniae* contamination on Sealwise WCB® anti-bacterial and standard Sealwise WCB® surfaces

Summary

Evaluation of the persistence of MRSA, *E. coli* and *K. pneumoniae* contamination (with or without soiling) on Sealwise WCB® anti-bacterial and standard Sealwise WCB® surfaces.

Date Collected: **November 2013**
Date Processed: **December 2013-February 2014**

Test(s) required: ***1. Survival of bacteria on Sealwise WCB® antibacterial and standard surfaces***
2. Transfer of contamination from test-surfaces to fingertips
3. Cleanability of contaminated test-surfaces

Reason for test: **Evaluation of efficacy of Sealwise WCB® antibacterial and standard Sealwise WCB® surfaces against bacteria likely to be present in the clinical (hospital) environment.**

Requested by: **Paul Huggins** (paul.huggins@sealwise.co.uk)
Managing director – Sealwise Ltd
Tel: +44 (0)1934750084

Client Details:
Sealwise Ltd
Unit 5 Mendip Business Park
Mendip Road
Axbridge
Somerset, BS26 2UG
www.sealwise.co.uk

Details of the products:

Antimicrobial surface
-***Description:*** Sealwise WCB® antibacterial surface
-***Active (antimicrobial) component*:** Silver (elemental)

Standard surface
-***Description:*** Sealwise WCB® standard surface
-***Active (antimicrobial) component*:** none

-***Manufacturer***: Sealwise Ltd
Unit 5 Mendip Business Park
Mendip Road
Axbridge
Somerset, BS26 2UG
www.sealwise.co.uk

Control surface
-***Description:*** Polypropylene-laminated wooden MDF surface
-***Active (antimicrobial) component*:** none

Methods

Summary of test conditions

-Target organisms:
i. **Methicillin-resistant *Staphylococcus aureus*** (MRSA); environmental isolate [pulse-type 15:B1]
ii. **Carbapenem-resistant *E.coli*** (CREC); clinical isolate
iii. Extended-spectrum-beta-lactamase (**ESBL**)-producing ***Klebsiella pneumoniae*; environmental isolate**

-Test surface A: Sealwise WCB® antibacterial surface

-Test surface B: Sealwise WCB® standard surface (non-antibacterial)

-Untreated (control) surface: Polypropylene-laminated wooden MDF surface (non-antimicrobial)

-Interfering substance (soil challenge):
- No soiling (sterile distilled water [DW])
- Heavy soiling (5% w/v Bovine Serum Albumin [BSA])

-Neutralising solution:
Tween 80 3% (w/v),
Lecithin 0.3% (w/v),
Sodium thiosulfate 1.0 % (w/v),
K_2HPO_4 1.5% (w/v),
KH_2PO_4 0.05% (w/v),
Poly-(sodium 4-styrenesulfonate) 1% (w/v)
Triton® X100 0.1% (v/v), and prepared in sterile PBS solution.

Experiments

1. **Experiment 1:** ***Effect of contact time on bacterial inoculum***

- A 1cm x 1cm square was marked on Sealwise WCB® antibacterial, Sealwise WCB® standard and an untreated Polypropylene (control) surface and each test area inoculated with 10uL of the test organism (CREC, MRSA or *K. pneumoniae*) suspended in a soil solution (Distilled water [DW] or 5% BSA).

- All surfaces were disinfected with 1000ppm NaDCC solution then scrubbed manually using a non-chlorinated detergent and rinsed three times using sterile distilled water (1Ltr). Once dry, cleaned surfaces were disinfected using 70% (v/v) ethanol solution and allowed to air-dry prior to use for experimentation.

- Inoculated surfaces were incubated at room temperature for contact times (t) of: 0, 1, 3, 24, 48 hours after which the test area was sampled with a cotton-tipped swab pre-moistened with neutralising solution.
  Swabs were transferred to 10mL (t=0, 1, 3hours) or 1mL (t=24, 48 hours) of neutralising solution and homogenised at high speed for 30 seconds by vortexing.

- Samples were incubated in neutralising solution for up to 10 minutes at room temperature (to allow neutralisation of antibacterial compound(s)). Serial dilutions of suspensions were performed (where appropriate) prior to spread-plating 0.1mL (t=0, 1, 3hours) or 0.5mL (t=24, 48 hours) aliquots onto Columbia blood agar plates. Plates were incubated aerobically at 37ºC for 48 h prior to reading.

2. **Experiment 2:** ***Transfer from surface to glove-tip***

- All test-surfaces were disinfected and prepared as previously described.

- A 1cm x 1cm test-area on treated and untreated (Polypropylene) surfaces was inoculated with 10uL of the test organism (CREC, MRSA or *K. pneumoniae*) suspended in a soil solution (Distilled water or 5% BSA). Inoculated test-areas were incubated at room temperature for 0 hours (wet inoculum) or 3 hours (dry inoculum).

- An unused, pristine nitrile glove (non-antibacterial) was donned by the operator (scientist) and the fingerpad of the index finger pressed (one second) onto a test-area inoculated with the test organism. The fingerpad of the contaminated glove was excised and transferred to a universal tube containing 10mL neutralising solution. Suspensions were homogenised by vortexing at high speed for 30 seconds and incubated a further 10 minutes at room temperature.

- Serial dilutions of suspensions were performed prior to plating on agar (described previously) and incubated aerobically at 37ºC for 48 h prior to reading.

3. Experiment 3: *Cleanability of Sealwise WCB® surfaces*

- All test-surfaces were disinfected and prepared as previously described.
- A 1cm x 1cm test-area on Sealwise WCB® antibacterial, Sealwise WCB® standard and an untreated Polypropylene (control) surface was inoculated with 10uL of the test organism (CREC, MRSA or *K. pneumoniae*) suspended in a soil solution (DW or 5% BSA). Inoculated test-areas were incubated at room temperature for 3 hours to allow the inoculum to dry.
- Inoculated surfaces were wiped clean using a microfiber cloth (10cm x10cm swatch) immersed in sterile tap water using two wiping motions (left-to-right, followed by right-to-left). Surfaces were sampled with a cotton-tipped swab, transferred to a universal tube containing 5mL neutralising solution and homogenised. The resulting suspension was plated on blood agar plates and incubated aerobically at 37^{o}C for 48 h prior to reading.

Results

Experiment 1: *Effect of contact time on bacterial inoculum*

Test areas were inoculated with between 1.3×10^3 - 6.56×10^3 (i.e. 3.11 $\log_{10}$ – 3.81 $\log_{10}$) colony-forming-units (cfu) bacteria.

The numbers of bacteria (MRSA, CREC and *K. pneumoniae*) remaining on untreated Polypropylene (control), and Sealwise WCB® anti-bacterial surfaces with increasing contact-time are shown in figure 1a, 1b and 1c respectively. Approximately 3 $\log_{10}$ cfu bacteria could be recovered from both treated and untreated (control) surfaces when sampled immediately (zero minutes contact time).

In the absence of soiling (DW only), between 1.6-2.1 $\log_{10}$ cfu of the contaminating bacteria remained on untreated (control) surfaces after 3 hours of contact time; these numbers fell to below the detection limit (2cfu) after 24 hours exposure. After 3 hours of contact time, treated (Sealwise WCB® anti-bacterial) surfaces were more effective at reducing the numbers of contaminating bacteria by approximately 1.5 $\log_{10}$ cfu than untreated surfaces if soiling is absent.

The presence of heavy soiling (5% BSA) allowed ~0.4 $\log_{10}$ cfu MRSA to persist for up to 48 hours and *K. pneumoniae* to persist for up to 24 hours. Although soiling reduced the efficacy of treated surfaces approximately 3 $\log_{10}$ cfu (>99.9%) reductions of these bacteria could be achieved and contamination reduced to below the detection limit (2cfu) in 24 hours.

Numbers of CREC fell to below the detection limit (2cfu) after 3 hours on treated surfaces when compared untreated surfaces (24 hours contact time). Soiling allowed CREC to persist at ~0.2 $\log_{10}$ cfu after 3 hours on Sealwise WCB® anti-bacterial surfaces but numbers fell to below the detection after 24 hours.

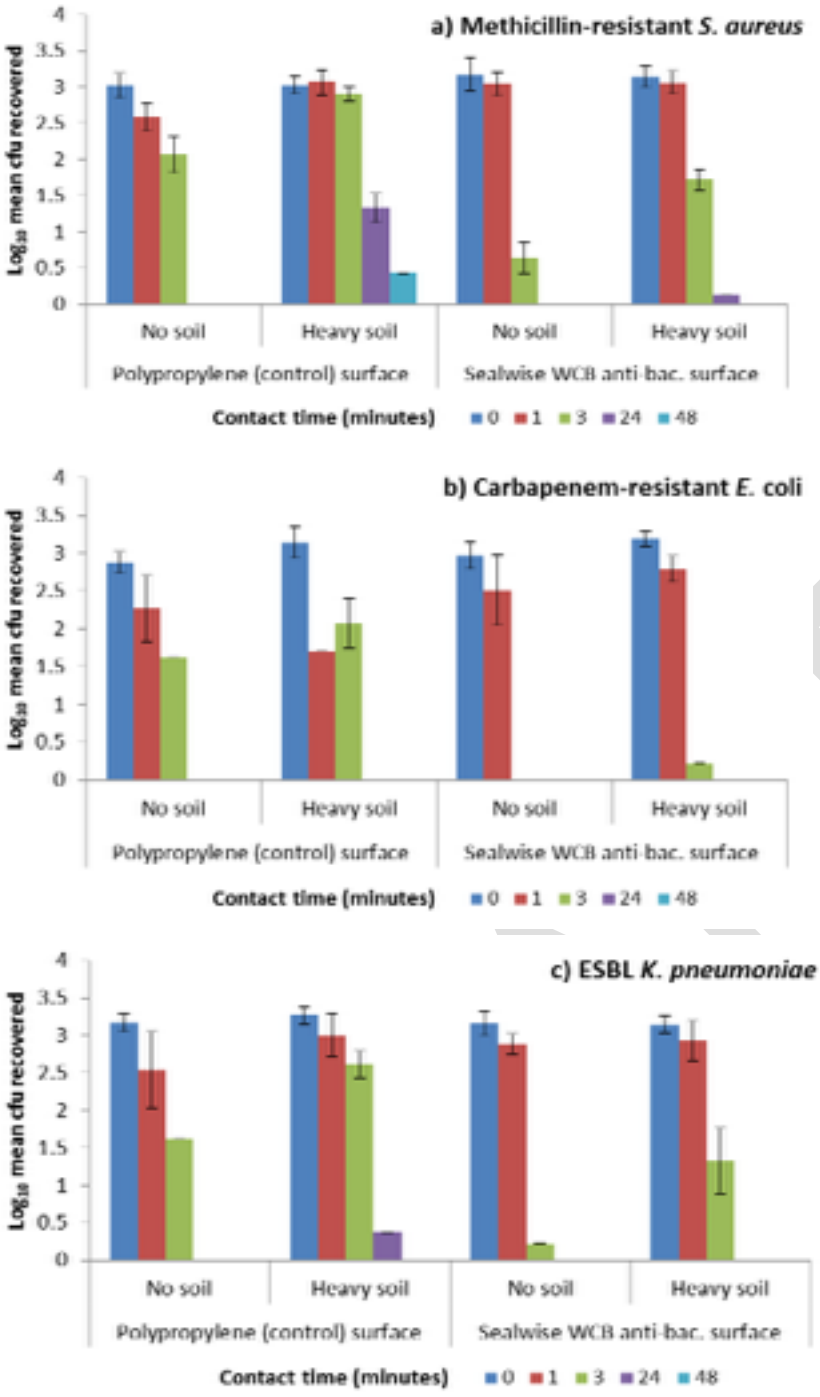

Figure 1c Effect of contact time on the numbers of bacteria recovered from treated (Sealwise WCB® anti-bacterial) and untreated (control) surfaces contaminated with a) MRSA, b) CREC or c) *K. pneumoniae* suspended in no soil or 5% BSA (heavy-soil conditions). The y-axis represents the mean number of bacteria (log_{10} cfu) remaining after increasing contact times on each surface (n=6); error bars indicate the standard deviation. Lowest detection limit is 2cfu ($0.30log_{10}$).

Experiment 2: *Transfer from surface to fingertips*

Surface were inoculated with between 1.3 x 10^3 - 6.56 x 10^3 (i.e. 3.11 $\log_{10}$ – 3.81 $\log_{10}$) colony-forming-units (cfu) bacteria and touched either immediately (wet inoculum) or after the inoculum was incubated on the surface for 3 hours (dry inoculum).

When surfaces were contaminated with wet inoculum of MRSA, between 2.5 $\log_{10}$ cfu (without soiling) and 3.5 $\log_{10}$ cfu (with heavy soiling) could be transferred from both all test-surfaces to an uncontaminated fingertip (figure 2a). Touching a dry inoculum present on polypropylene (control) surfaces, transferred between 0.9 $\log_{10}$ cfu (without soil) and 1.4 $\log_{10}$ cfu MRSA (with heavy soiling) to fingertips. Both Sealwise WCB® standard and Sealwise WCB® antibacterial-treated surfaces contaminated with dry inoculum of MRSA transferred higher numbers of MRSA (~1.0-1.9 $\log_{10}$ cfu) to fingertips whether soiling was present or absent.

Regardless of soiling, touching either test-surface contaminated with CREC transferred ~3 $\log_{10}$ cfu bacteria to fingertips when the inoculum was wet; however, numbers of CREC detected on fingertips when touching a surface contaminated with a dry inoculum was <1 cfu (figure 2b).

Fingertips could be contaminated with between 3.5-3.8 $\log_{10}$ cfu *K. pneumoniae* when touching a wet inoculum on all test-surfaces; soiling had no effect on the numbers of bacteria transferred from wet inoculum to fingers (figure 2c). Touching the same surfaces when a dry inoculum was present transferred low numbers (<1 cfu) *K. pneumoniae* to fingertips but ~0.5 $\log_{10}$ cfu when touching antibacterial surfaces that were heavily soiled.

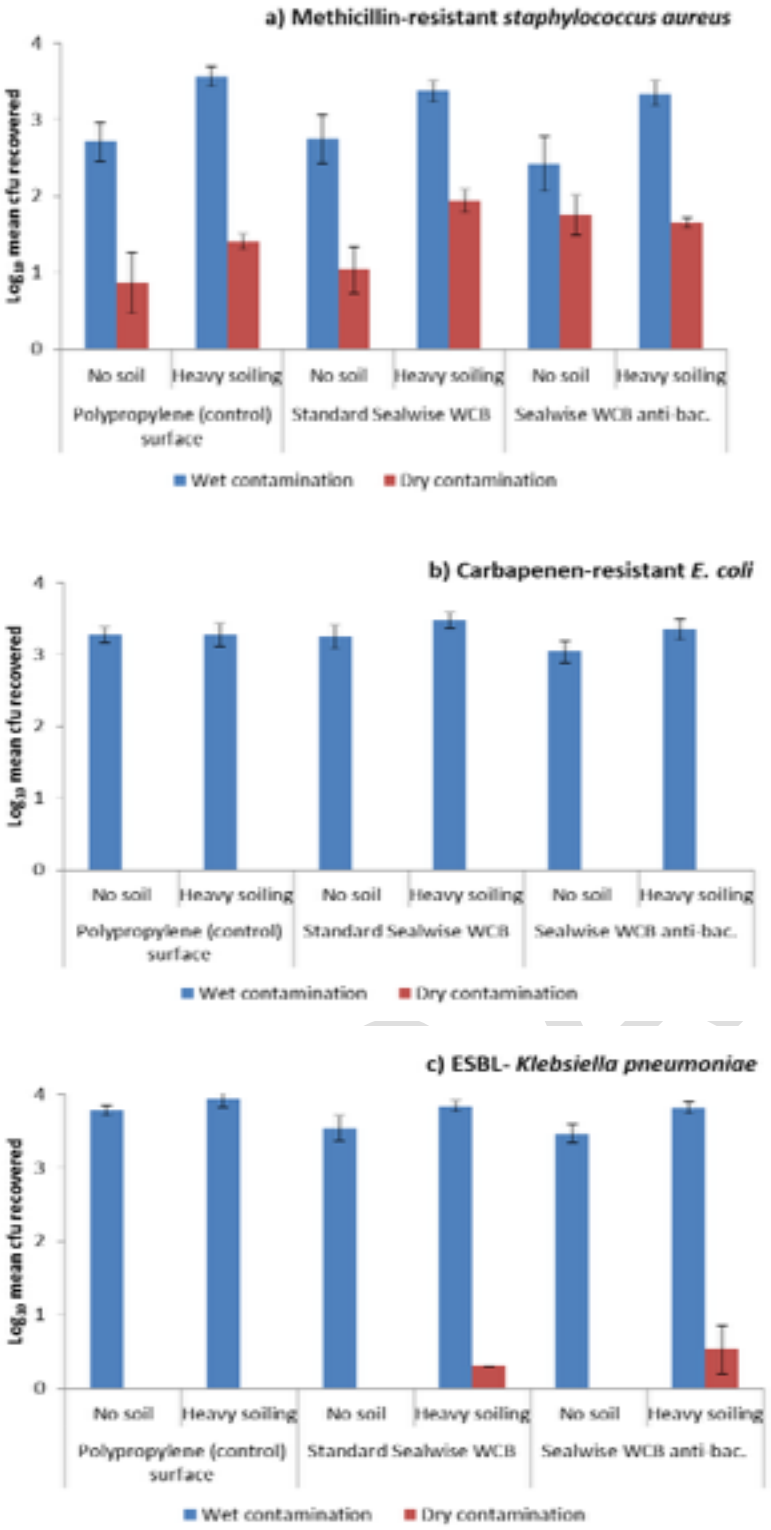

Figure 2 Numbers a) MRSA, b) CREC or c) *K. pneumoniae* transferred to fingertips when touching untreated Polypropylene (control), standard Sealwise WCB® and Sealwise WCB® anti-bacterial surfaces with or without heavy-soil conditions. The y-axis represents the mean number of bacteria (log_{10} cfu) transferred from wet and dry contaminated fingertips (n=6); error bars indicate the standard deviation. Lowest detection limit is 2cfu (0.30 log_{10}).

Experiment 3: *Persistence of bacteria on treated and untreated surfaces post-cleaning*

Sealwise WCB® antibacterial, Sealwise WCB® standard and untreated (control) surfaces contaminated with dry inoculum (3 $\log_{10}$cfu of bacterial suspensions) with or without heavy soiling were cleaned with a microfiber cloth wetted with tap water only. The persistence of bacteria post-cleaning is displayed in figures 3a-3b.

When no soils were present, the polypropylene (control) surfaces could be cleaned effectively with a microfiber cloth, reducing numbers of MRSA, CREC and *K. pneumoniae* to below the detection limit (2cfu) but proved more difficult to clean when heavily soiled. Approximately 1.1 $\log_{10}$cfu (MRSA), 1.1 $\log_{10}$cfu (CREC) and 0.2 $\log_{10}$cfu (*K. pneumoniae*) persisted on the polypropylene (control) surfaces post-cleaning.

Regardless of soiling, MRSA, CREC and *K. pneumoniae* could not be detected (i.e. <2cfu) on either Sealwise WCB® antibacterial or Sealwise WCB® standard surface after cleaning with a microfiber cloth.

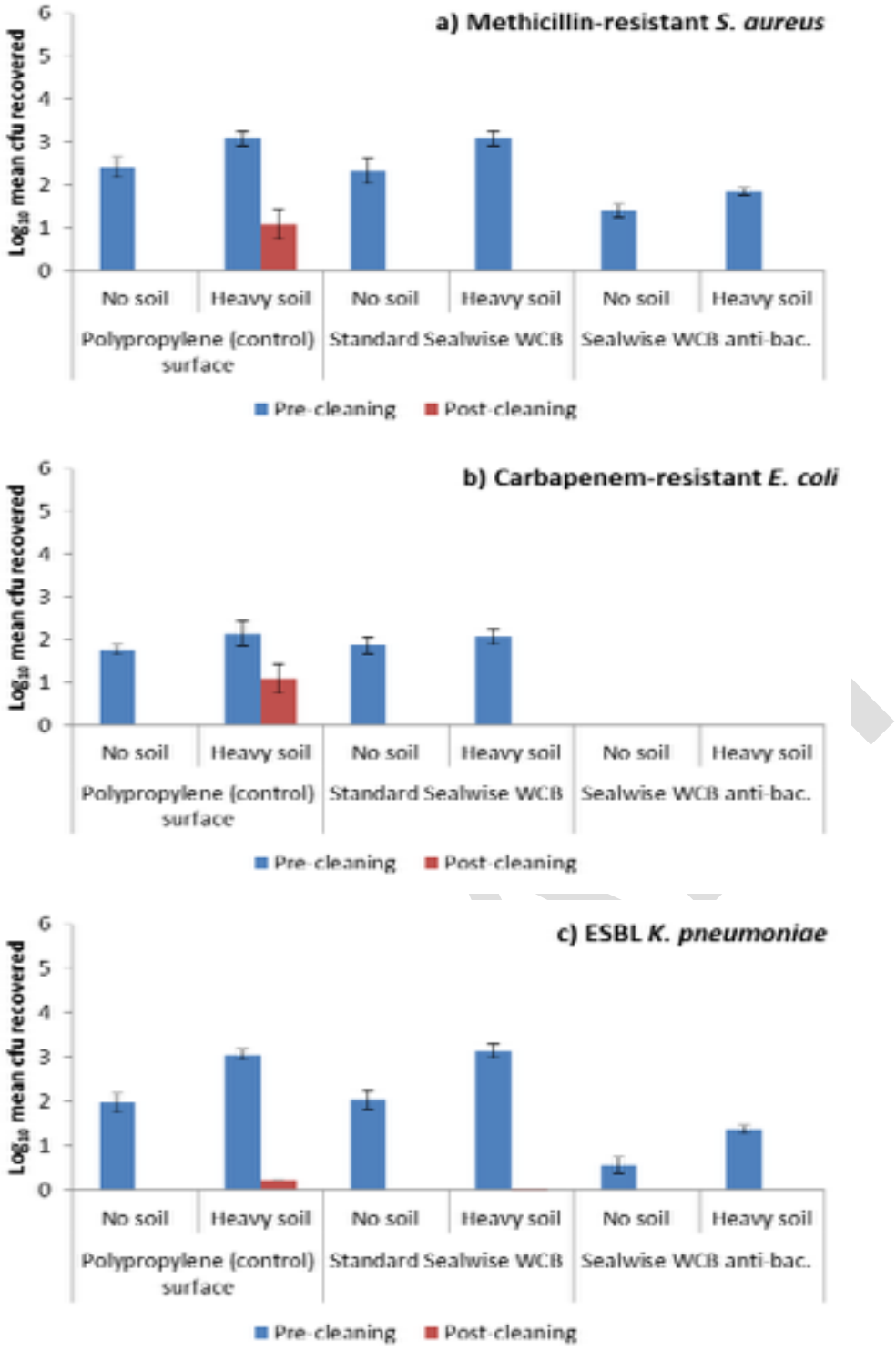

Figure 3c Number of bacteria persisting after cleaning untreated Polypropylene (control), standard Sealwise WCB® and Sealwise WCB® anti-bacterial surfaces contaminated with a) MRSA, b) CREC or c) *K. pneumoniae* with or without heavy soiling. The y-axis represents the mean number of bacteria (log_{10} cfu) persisting on surfaces before and after cleaning with a microfiber cloth wetted with tap water (n=6); error bars indicate the standard deviation. Lowest detection limit is 2cfu (0.30 log_{10}).

Discussion and Conclusions

Methicillin-resistant *Staphylococcus aureus* (MRSA); pulse-type 15:B1, carbapenem-resistant *E. coli* (CREC) and extended-spectrum-beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* are clinically-significant pathogens responsible for hospital-acquired infections in humans. Sealwise WCB® antibacterial surfaces demonstrated antimicrobial activity against MRSA, CREC), and an ESBL-producing *Klebsiella pneumoniae*.

In the absence of any cleaning (mechanical) action, Sealwise WCB® antibacterial surfaces can reduce contamination levels of MRSA, CREC and *K. pneumoniae* by >3 $\log_{10}$ cfu (99.9% reductions) to below the detection limit (2 cfu) within 24 hours of even when heavily soiled with dirt. This demonstrates a potential for Sealwise WCB® antibacterial surfaces to reduce the risk of cross-contamination of uncontaminated surfaces and hands with clinically-significant bacteria.

Recently contaminated surfaces (wet inocula) and surfaces contaminated with dry inocula pose a risk of cross-contaminating uncontaminated hands with bacteria. Sealwise WCB® standard and Sealwise WCB® antibacterial surfaces were noted to exhibit hydrophobicity against the bacterial inoculum causing the inocula to form beads on the surface. This characteristic may promote the ability of the Sealwise WCB® surfaces to "shed" bacteria when touched or manually cleaned. This was demonstrated by the propensity of Sealwise WCB® surfaces to transfer greater numbers of MRSA and *K. pneumoniae* to fingertips than the polypropylene-laminated MDF surfaces. Furthermore, Sealwise WCB® standard and Sealwise WCB® antibacterial surfaces could also be cleaned more effectively than the polypropylene surfaces.

Furniture in the patient vicinity, such as bedside cabinets, may become highly contaminated with bacteria if touched frequently by an infected patient. Bacteria may also harbour in difficult-to-clean sites such as gaps in joints between panels that form standard cabinet frames. Effective decontamination of these surfaces is essential in reducing the bioburden and subsequent cross-contamination to other surfaces. Sealwise WCB® cabinets are produced by a joining process using tetrahydrofuran to permanently seal panels together and create homogenous edges that prevent the formation of difficult-to-clean gaps. Therefore, the use of easy-to-clean surfaces such as the Sealwise WCB® standard and Sealwise WCB® antibacterial surfaces to create hospital furniture would be valuable in the clinical setting.

Notes / disclaimer
The use of Sealwise WCB® antibacterial surfaces may be beneficial in the clinical environment where the infection control practices are of paramount importance. However, the antimicrobial activity of Sealwise WCB® antibacterial surfaces against MRSA, CREC and *K. pneumoniae*, cannot be relied-upon solely for the control/reduction of infectious organisms in the clinical environment as soiling (e.g. BSA) will significantly reduce the efficacy of such materials. The use of antimicrobial materials such as Sealwise WCB® antibacterial surfaces should be utilised in conjunction with good cleaning and hand hygiene practices and surfaces should be decontaminated frequently.

-End of report-

Dr Shanom Ali

Scientist,
Environmental Research Laboratory,
University College Hospital**,**
London, NW1 2BU

Prof. Peter Wilson, MA MD FRCP FRCPath
Consultant Microbiologist,
Clinical Microbiology & Virology,
UCLH NHS Foundation Trust,
London W1T 4EU

Please note:

This report does not constitute endorsement of the tested products in any way by the authors/scientists involved in the production of this report or by the UCLH Environmental Research Laboratory or by any hospital belonging to the University College London Hospitals NHS Foundation Trust. The names of authors of this report or 'Environmental Research laboratory' or 'University College London Hospitals NHS Foundation Trust' may not be used in any type of promotional published material, either written or electronic, without prior consent and written permission from both parties.