Title: Contamination of Clinical White Coats with Potential Pathogens and their Antibiotic Resistant Phenotypes Among a Group of Sri Lankan Medical Students

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Discussion Points: The work described here looks at the possibility of the clinical white coats of medical students being contaminated with potential pathogens and their resistant phenotypes, highlighting the importance of their role as potential sources of infections #AMR, #medical_students, #srilanka #lka, #whitecoats, #antibioticresistance.

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ABSTRACT. Up to 250 words (249 words)

Background: Clinical white coats worn by the medical students can be contaminated at hospitals and act as a potential reservoir for pathogens including antibiotic-resistant bacteria. This study aimed to identify the contamination rates of clinical white coats worn by medical students with selected potential pathogens and their antibiotic resistant phenotypes.

Methods: A cross-sectional study was done among 151 4th year medical students of Faculty of Medicine, University of Peradeniya, Sri Lanka in September 2020. The participants belonged to two batches undergoing clinical training at two settings. Swabs from pockets and sleeves of the clinical white coats were taken. Potential pathogens and their resistant phenotypes were identified with routine tests.

Results: Fifty-three participants (35.1%) had coats contaminated with *Staphylococcus aureus*; 15 (9.9%) had coats contaminated with Methicillin-Resistant *S.aureus* (MRSA). One *Enterobacterales* (0.7%) was an *AmpC* producer. *Enterococcus* species were isolated from 19 (12.6%) coats and 2 (1.3%) had coats contaminated with vancomycin resistant enterococci. Molecular testing on the MRSA isolates identified that 5(20%) of the MRSA isolates were *PVL* positive while all were *mecA* positive. Sex, type of clinical appointment, and frequency of washing white coats were not associated with contamination. The “batch” was significantly associated with contamination with *S.aureus* and *Enterococcus* species.

Conclusions: We found that clinical white coats worn by medical students recruited for the study were contaminated with *S.aureus*, MRSA and *Enterococcus* species. There was a notably high-rate of contamination with *S. aureus.* All MRSA isolates were mecA positive while the rate of *PVL* positivity was low.

Key Words: *Drug Resistance, Microbial; Infection control; Microbiology; Students medical*

INTRODUCTION.

Clinical white coats are worn by health-care workers including clinicians and medical students in many countries. While most developed countries have moved away from clinical white coats to scrubs, white coats remain part of the hospital attire in many developing countries including Sri Lanka.

Clinical white coats, however, are considered to be possible vehicles for transmission of pathogens.[1] Microorganisms may live on the fabric of clinical coats for several days, even up to three months.[2] Therefore, these can act as potential reservoirs for the transmission of antibiotic resistant bacteria. Medical students spend long hours in different clinical settings as per their training requirements, being in different venues such as wards, clinics, and in-hospital teaching areas in the same attire. Therefore, contamination of their white coats can contribute to horizontal transmission of potential pathogens from patient to patient as well as the spread of those to different physical areas within health-care institutes. This could also lead to an increase in the rates of health-care associated infections, including those caused by antibiotic resistant bacteria. Further, this may also contribute to the spread of antibiotic resistant bacteria to the community.

This study aimed to describe the patterns of contamination of white coats among medical students in a Sri Lankan medical school, with selected potential pathogens and their antibiotic resistant phenotypes; namely, *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Enterobacterales* species, Extended Spectrum Beta lactamase (ESBL) producing *Enterobacterales* and *AmpC* producing *Enterobacterales*, *Enterococcus* species and vancomycin resistant *Enterococcus* (VRE) species.

METHODS

This cross-sectional study conducted was among 4th year medical students of Faculty of Medicine, University of Peradeniya, Sri Lanka in September 2020, before the attire of medical students were changed to scrubs. The study site had two 4th year batches (Batch A and B). Clinical training for the two batches were conducted predominantly in two institutes at the time of the study, Teaching Hospital, Peradeniya, Sri Lanka and National Hospital, Kandy. Sri Lanka. All except those who were wearing short or three-quarter sleeved white coats were eligible to participate in the study. A self-administered data collection sheet was used to gather demographic data, current clinical appointment, predominant method of wearing the sleeve of the coat (rolled up or not), frequency of washing the coat, the date of last washing of the coat and wearer's perception on cleanliness of their white-coat. A pre-test was done on 10 medical students of the 3rd year batch before implementing the study proper. Ethics approval was obtained from the ethics review committee of Faculty of Medicine, University of Peradeniya, Sri Lanka (2020/EC/SP/01). Informed, written consent was obtained from participants.

Two sterile swabs, moistened with sterile 0.9% saline were used to obtain samples from pockets and the cuffs of sleeves of each lab-coat as these are the sites frequently handled by the wearers and come in to contact with patients, respectively. Swabs were collected by the investigators adhering to a pre-planned protocol, with measures to prevent cross-contamination, inserted to individual plastic sheaths, transported to the lab immediately and inoculated in 10 ml of Brain Hearn Infusion (BHI) broth (Oxoid, UK). BHI broth was incubated overnight at 37°C. The next day, 10 µl each was plated on a Mannitol Salt agar (MSA) (Oxoid, UK) plate and a MacConkey agar (Oxoid, UK) plate supplemented with Cefotaxime at 1 µg/ml (HiMedia, India) concentration to screen for potential *Enterobacterales* and a Chromogenic agar plate (BioMaxima, Poland) to identify potential *Enterobacterales* and *Enterococcus* species. All plates were prepared according to manufacturer's instructions. Potential isolates were picked, and identified using routine biochemical testing [3].

Staphylococcus aureus isolates were tested for sensitivity to cefoxitin to identify MRSA and Methicillin Sensitive *Staphylococcus aureus* (MSSA) isolates. Sensitivity to cefotaxime and ceftazidime were checked in the *Enterobacterales* species to screen for possible ESBL producers. *Enterobacterales* species fulfilling the criteria for potential ESBL producers were subjected combined disc testing and were also tested for *AmpC* production with disc diffusion method (Mast, UK). Sensitivity to other relevant antibiotics were tested according to the CLSI guidelines. [4-6] Enterococci was tested for sensitivity to ampicillin with disc diffusion method and minimum inhibitory concentration (MIC) for vancomycin (Sigma-Aldrich, Singapore) was tested using macro-broth dilution method.[7]

DNA was extracted from the 20 MRSA isolates by boil lysis and presence of *PVL* and *mecA* genes were assessed by previously established conventional PCR.[8,9]

For analysis, medicine, pediatrics, and psychiatry appointments were groups together as medical appointments while surgery, gynaecology and obstetrics and other surgical sub-specialties were grouped together as surgical appointments. Wearers' perception on the cleanliness of the coat was thematically analyzed. Two themes emerged as clean and contaminated, thereafter, these two themes was used as a binary variable in further analysis.

In data analysis, percentages were calculated for contamination of white coats in each site with the selected potential pathogens and their antibiotic resistant phenotypes. The Chi-square's test or Fishers Exact test were used to test for associations while the Mann-Whitney U test was used to compare the differences in continuous

variable. A p-value of less than 0.05 was considered as statistically significant. All analysis was done on SPSS (IBM) version 21.

RESULTS.

A total of 151 participants were recruited. Of this, 72 (47.7%) were from 4th year batch A and 79 (53.3%) were from 4th year batch B.

The numbers of female and male students were 78(51.7%) and 73 (48.3%) respectively. The mean number of days from last washing to sample collection was 6.2 (SD 5.8) while the median was 4.0 (IQR 3 – 7) days. Other parameters stratified in relation to the two batches are given in Table 1..

Among the 151 participants, *Staphylococcus aureus* was isolated from one or both swabs in 53 (35.1%) participants. Coats of 15 of the 151 (9.9 %) participants were contaminated with Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates. Twelve (7.9%) of the coats were contaminated with bacteria of the order *Enterobacterales* with the tests used. None of the isolates were found to be ESBL producers; however, one (0.7%) coat was contaminated with an *AmpC* producing *Enterobacterales* species. Nineteen (12.6%) participants had coats that were contaminated with *Enterococcus* species and two (1.3%) participants had coats contaminated with VRE (Table 2). The differences in contamination rates between pockets and sleeves were not statistically significant.

At least one potential pathogen of interest was found to contaminate 74 (49.0%) of the coats while 18 coats (11.9%) were contaminated with at least one of the resistant phenotypes of interest (MRSA, VRE or AmpC producers). Ten (6.6%) coats were contaminated with two types of potential pathogens while 64 (42.4%) were contaminated with only one type.

Contamination rates with *Staphylococcus aureus* and *Enterococcus* species was significantly different between the two 4th year batches. Other parameters analyzed in relation to contamination with the selected bacterial contaminations did not differ significantly and are given in Table 3.

Association between the pattern of wearing the sleave (rolled up vs left long) and contamination with resistant bacteria at the coat sleeve was assessed. There was no significant association between rolling up the sleeves and colonization with *S. aureus* (18.8% vs 34.8%), MRSA (7.0% vs 8.7%) or *Enterococcus* species (6.3% vs 8.7%) ($p>0.05$, Fisher's Exact test).

Among the 53 white coats contaminated with *Staphylococcus aureus,* 19 were contaminated only at sleeves of coats, 21 only at pockets of coats and 13 at both sites; leading to a total of 66 *Staphylococcus aureus* isolates. Among the *Staphylococcus aureus* isolates, 20 (30.30%) were MRSA while 46 (69.67%) were Methicillin Sensitive *Staphylococcus aureus* (MSSA). The susceptibility rates for different antibiotics were higher among the MSSA isolates than the MRSA isolates except for ciprofloxacin; [gentamycin (95.7% vs 85%), ciprofloxacin (50% vs 70%), clindamycin (91.3% vs 70.0%), erythromycin (58.7% vs 45%) and tetracycline (95.7% vs 90%)] Molecular testing on the MRSA isolates identified that five of the 20 MRSA isolates were *PVL* positive while all were *mecA* positive.

Of the 20 *Enterococcus* isolates four were ampicillin resistant, two isolates were identified as VRE.

DISCUSSION.

The current study aimed to describe the pattern of contamination of clinical white coats with selected antibiotic resistant bacteria. *Staphylococcus aureus* was isolated from swabs taken from 53 participants (35.1%). Out of these, 53 participants with *Staphylococcus aureus* contaminating white coats, 15 were contaminated with MRSA (9.9%). All the MRSA isolates were positive for *mecA* gene while only 5 were positive for *PVL* gene.

A number of studies had previously assessed the rates of *Staphylococcus aureus* contamination in clinical white coats. Despite varying in frequency of contamination, *Staphylococcus aureus* has been identified as the commonest isolate contaminating clinical white coats in many studies.[1,10,11,12] Similarly, MRSA isolation rates from white coats ranged from 3.5%[13] to up to 79% (during outbreaks of infections in units).[14] These differences in contamination rates could be due to differences in institutional environments, infection prevention and control measures as well as wearer habits such as performing hand hygiene.

While contamination rates of clinical white coats are not available for Sri Lanka, Munasinghe *et al* has reported a colonization rate of 22.0% and 4.3% for *Staphylococcus aureus* and MRSA from nasal swabs obtained from a group of university students of the same study site.[9] In addition in the same study, 21.4% of the identified MRSA were found to be *PVL* positive. We did not assess to see if the wearers of the coats were colonized with any of the pathogens tested for. However, using the *PVL* positivity rate, it is clear that most of the isolates obtained from the white coats are of hospital origin as the *PVL* positivity rates were lower in the current study when compared to the colonization study, as *PVL* which is a virulence factor in MRSA is more commonly found in isolates of community origin. All isolates were found to contain *mecA. MecA* gene codes for an altered in the penicillin binding protein leading to resistance in beta-lactam drugs. [15,16]

Nineteen (12.6%) participants of the present study had coats that were contaminated with *Enterococcus* species and two of them (1.3%) were vancomycin resistant. A study by C. Kannangara *et al*. has shown that vancomycin resistant enterococci (VRE) rectal colonization rate of 5% among 218 patients in an intensive care unit of the National Hospital of Colombo, Sri Lanka.[17] It is obvious that VRE isolates are circulating in health-care settings in Sri Lanka and that contaminated cloths may act as a vehicle of transmission. Given the possibility of horizontal gene transfer for vancomycin resistance, this is a concerning situation.

Twelve (7.9%) of the coats of participants were contaminated with *Enterobacterales* and none of the isolates were found to be ESBL producers. However, one (0.7%) coat was contaminated with an *AmpC* producing *Enterobacterales* species. A study done at Kilimanjaro Christen Medical Center in Tanzania, found that 3 out of 180 coats were contaminated with *E. coli*.[12] This, and other studies indicate that contamination of clinical white coats with Gram negative isolates is relatively uncommon than contamination with Gram positive isolates. While our study did not identify any ESBL producers to contaminate clinical white coats, ESBL producers are common in Sri Lankan hospitals and the community, both as causative agents for infections and as colonizers.[9,18] However, clinical white coats are usually dry environments and Gram negatives do not usually thrive in such conditions unlike *Staphylococcus aureus* or *Enterococcus* spp.

In this study rate of contamination of clinical white coats with *S. aureus*, MRSA, *Enterobacterales* and *Enterococcus* was assessed in relation to several variables such as sex, batch, current clinical appointment, frequency of washing, and one's perception of cleanliness of his/her clinical white coats. Out of these variables, only the batch was found to have a statistically significant association with the rate of contamination. Batch A had a significantly higher contamination rate with *Enterococcus* species while batch B has a higher rate of

contamination with *Staphylococcus aureus*. At the time of the study, the two batches had their clinical training at two hospitals, where the predominant environmental contaminants may be different and this may explain the difference in contamination rates. Further, the frequency of washing coats significantly differed between the two batches, however, the impact of this on the association with different potential pathogen remains to be explored further. It is of interest to note that the frequency of washing and the duration since the last wash was not significantly associated with contamination rates.

One major disadvantage of this study is that we did not look at the contamination with *Clostridium difficile*. This was not possible as the study site lacked anaerobic culture facilities. Further, we selected these isolates to focus on as funding limitations prevented us from focusing on all ESKAPE pathogens

In conclusion, we have identified a considerable high rate of contamination with potential pathogens, particularly Gram positive isolates. This is a reason for concern. Further, association of *Staphylococcus aureus* and *Enterococcus* species with the batches, where the main difference was the hospital where they were trained, indicates that hospital environment may play a bigger role on this.

The current study highlights the importance of establishing a mechanism to ensure that attire worn in health-care settings, are cleaned frequently. While our study population is from a single university, in the current times of global travel, these findings are of global concern. With the escalation of the COVID-19 pandemic, Sri Lankan universities transitioned from white coats to scrubs as the attire for medical students. We hope this would have had a positive impact on the possible contamination with potential pathogens as scrubs are worn next to skin, and therefore, unlike white coats are likely to be washed more frequently.

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SUMMARY - ACCELERATING TRANSLATION

Contamination of white coats with germs

Clinical white coats were going out of practice in many countries but in some countries like Sri Lanka it continued to be a part of the attire of medical students till the emergence of COVID-19 pandemic. In this study we took samples from 151 white coats worn by medical students attached to the Faculty of Medicine, University of Peradeniya, Sri Lanka and tested to see if any germs causing infections are found in those. Regular laboratory methods were used in the testing and samples were obtained from the cuffs and pockets of the white coats. We identified three types of infection causing germs in the coats. We also found two types of germs that are resistant to antibiotics, namely methicillin resistant *Staphylococcus aureus* (MRSA) in 15 (9.9%) coats and vancomycin resistant enterococci (VRE) 2 (1.3%) in two. We emphasize the importance of having strict guidelines to ensure that those who wear white coats including medical students clean them more frequently so that their role as potential reservoirs of germs may be lessened.

FIGURES AND TABLES.

Table 1: Description of the Study Population

Variables		All participants n (%)	Batch A	Batch B	Difference
Sex	Male	73 (48.3 %)	40 (55.6%)	33 (41.8%)	0.10
	Female	78 (51.7 %)	32 (44.4%)	46 (58.2%)	
Appointment[1]	Medical	86 (57 %)	47 (65.3%)	39 (49.4%)	0.07
	Surgical	65 (43 %)	25 (34.7%)	40 (50.6%)	
Frequency of washing[2]	< 1 once a week	118 (78.1 %)	62 (86.1%)	56 (70.9%)	0.03*
	> Once a week	33 (21.8 %)	10 (13.9%)	23 (29.1%)	
Perception[2]	Clean	30 (24 %)	12 (18.5%)	18 (30.0%)	0.147
	Contaminated	95 (76 %)	53 (81.5%)	42 (70.0%)	
Time since washing the coat	Median (IQR)	4.0 (3 – 7)	3 (3 – 5)	4 (3- 10)	0.003^

1 – For analytical purposes medicine, pediatrics and psychiatry considered as medical appointments while surgery, gynaecology and obstetrics and other surgical sub-specialties were considered as Surgical appointments

2- Only 125 (83.8%) participants presented their perception on cleanliness of the coats

* Chi-square test, ^ Mann-Whitney U test

Table 2- Summery of Colonization Rates

Organism	Sleeves only N (%)	Pockets only N(%)	Both N(%)	Either of the sites or both N(%)
Staphylococcus aureus	19 (12.6%)	21 (13.9%)	13 (8.6%)	53 (35.1%)
MRSA	6 (4%)	4 (2.6%)	5 (3.3%)	15 (9.9%)
Enterobacterales species	5 (3.3%)	5 (3.3%)	2 (1.3%)	12 (7.9%)
ESBL producers	0	0	0	0
AmpC producers	0	1 (0.7%)	0	1 (0.7%)
Enterococcus species	9 (6.0%)	9 (6.0%)	1 (0.7%)	19 (12.6%)
VRE	0	2 (1.3%)	0	2 (1.3%)

Table 3 - Association of Variables Studied with Contamination of Coats

Variable		Contamination with *Staphylococcus aureus*			Contamination with MRSA			Contamination with *Enterococcus* spp		
		Contamination absent	Contamination present	Significance	Contamination absent	Contamination present	Significance	Contamination absent	Contamination present	Significance
Sex	Male (n=73)	52 (71.2 %)	21 (28.8 %)	0.11	64 (87.7 %)	9 (12.3 %)	0.34	62 (84.9%)	11 (15.1%)	0.37
	Female (n=78)	46 (59.0 %)	32 (41.0 %)		72 (92.3 %)	6 (7.7%)		70 (89.7%)	8 (10.3%)	
Batch	4th year batch A (n=72)	53 (73.6 %)	19 (26.4 %)	**0.03***	66 (91.7 %)	6 (8.3%)	0.53	56 (77.8%)	16 (22.2%)	**0.001***
	4th year batch B (n=79)	45 (57.0 %)	34 (43.0 %)		70 (88.6 %)	9 (11.4%)		76 (96.2%)	3 (3.8%)	
Appointment	Medical (n=86)	58 (67.4%)	28 (32.6%)	0.45	78 (90.7%)	8 (9.3%)	0.77	76 (88.4%)	10 (11.6%)	0.68
	Surgical (n=65)	40 (61.5 %)	25 (38.5 %)		58 (89.2 %)	7 (10.8 %)		56 (86.2%)	9(13.8%)	
Frequency of washing	<=1 once a week (n=118)	78 (66.1 %)	40 (33.9 %)	0.56	107 (90.7 %)	11 (9.3 %)	0.74	102 (86.4%)	16 (13.6%)	0.77
	> Once a week (n=33)	20 (60.6 %)	13 (39.4 %)		29 (87.9 %)	4 (12.1 %)		30 (90.9%)	3 (9.1%)	
Perception	Clean (n=30)	20 (66.7 %)	10 (33.3%)	0.86	26 (86.7 %)	4 (13.3%)	0.29	29 (96.7%)	1 (3.3%)	0.07
	Contaminated (n=95)	65 (68.4 %)	30 (31.6%)		88 (92.6 %)	7 (7.4%)		78 (82.1%)	17 (17.9%)	
Time since coats were cleaned	Mean (SD)	6.51 (6.6)	5.61 (3.9)	0.62	6.1 (5.9)	7.4 (4.5)	0.07	6.22 (6.0)	6.05 (4.4)	0.94
	Median (IQR)	3.5 (3 – 8)	4 (3 – 7)		4 (3 – 6)	5 (3 – 11)		4 (3 – 7)	3 (3 – 8.5)	

* Chi-square test