High Touch Hospital Surfaces as Reservoirs of Staphylococci with Elevated Oxacillin Screening Minimum Inhibitory Concentrations

A Perera [1], W Kudagammana[2], R Dissanayake R[2], N Handapangoda[2], R Zheng[3], J Yang[3], WS Lee[3], N Barua[3], M Ip[3,4,5,6], V Liyanapathirana[2]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Science, University of Peradeniya, Sri Lanka
[2]Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka
[3]Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, People's Republic of China
[4]Prince of Wales Hospital, Shatin, N.T., Hong Kong SAR, People's Republic of China
[5] S.H.Ho Research Centre for Infectious Diseases, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, People's Republic of China
[6]Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, People's Republic of China

Background

- Healthcare-associated infections by antibiotic-resistant staphylococci -> global health threat
- High-touch surfaces -> act as critical reservoirs -> transmitting these pathogens
- Despite the increasing infection burden, the contribution of surface contamination is under-researched.
- Data on isolates with elevated oxacillin screening minimum inhibitory concentrations in hospital settings are limited.

Objectives

1. To determine the frequency of *Staphylococcus aureus*, methicillin-resistant *S. aureus* and Coagulase-negative staphylococci (CoNS) contamination on high-touch surfaces at Teaching Hospital, Peradeniya and to evaluate the association factors for contamination
2. To screen the isolates for elevated Oxacillin minimum inhibitory concentration (MIC) and to characterize antibiotic resistance pattern in *S. aureus* phenotypically and genotypically

Methodology

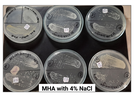
- A cross-sectional study
- High Touch Surfaces at Teaching Hospital, Peradeniya
- Sample collection period - February to May 2025
- Hospital units sampled:
  Intensive Care Unit (ICU)
  Outpatient Department (OPD)
  Clinical Complex
  Surgical Wards (Male and Female)
- Sample size: 212

Sample Collection

Selective Enrichment

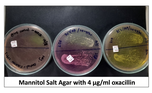
- Incubation at 35℃ for 18-24hrs

Isolation on Media



- Incubation at 35℃ for 18-24 hrs

Screening for Oxacillin MIC



- Incubation at 35℃ up to 24 - 48hrs

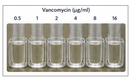
Phenotypic Identification of *S. aureus* and CoNS

- Gram Stain
- Catalase Test
- Coagulase Test
- DNase Test

Antibiotic Sensitivity Test (Kirby - Bauer Disk Diffusion Method)

- FOX - Cefoxitin (30µg)
- DA - Clindamycin (2µg)
- E - Erythromycin (15µg)
- CN - Gentamicin (10µg)
- CIP - Ciprofloxacin (5µg)

Determination of Vancomycin MIC (Broth Microdilution Method)

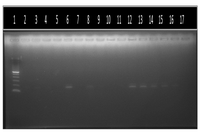


- Conducted according to the CLSI guideline

Determination of presence of *mecA* gene (PCR Method)

Constituent	Concentration	Per Reaction Volume (µL)
GoTaq Green Master mixer (Promega, USA)	2X	12.5
Forward primer - *mecA*	10 µM	1
Reverse primer - *mecA*	10 µM	1
Molecular grade water		8.5
DNA template		2

Thermal Cycler Conditions	No. of Cycles	Temperature	Time Duration
Initial denaturation		95 °C	2 minutes
Denaturation	30	95°C	1 minute
Annealing		59 °C	1 minute
Extension		72 °C	1 minute
Final extension		72 °C	5 minutes



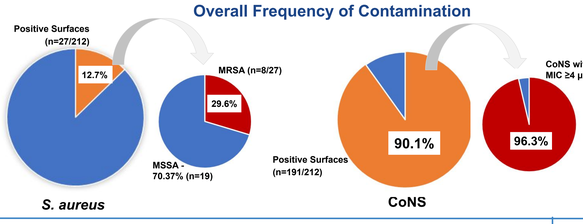
1	Ladder	10	MHA 113A
2	MRSA 32A	11	MHA 160D
3	MHA 46A	12	PC1
4	MHA 63A	13	PC2
5	MRSA 63A	14	PC3
6	MRSA 85A	15	PC4
7	MHA 85A	16	PC5
8	MHA 86A	17	NC
9	MHA 89A		

Primer sequences used for *mecA* detection were as follows:

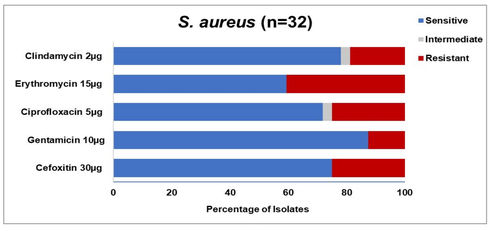
- *mecA* P4 2821 5'- TCCAGATTACAACTTCACCAGG -3'
- *mecA* P7 2822 5'- CCACTTCATATCTTGTAACG -3'

Results

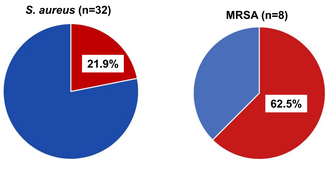
Overall Frequency of Contamination



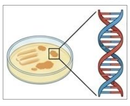
Characterization of Antibiotic Resistance



Multi Drug Resistance among Isolates



Presence of *mecA* Gene in MRSA Isolates



***mecA* positive (25%, n=2/8)**

***mecA* negative (75%, n=6/8)**

MIC against Vancomycin in MRSA Isolates (n=8)

Vancomycin MIC (mg/dL)	n(%)
1 µg/ml	7 (87.5%)
2 µg/ml	1 (12.5%)

- **No VISA or VRSA** strains were detected.

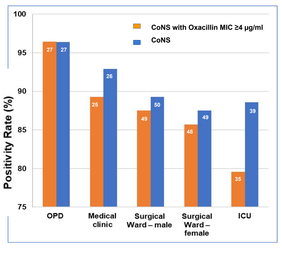
Association Factor Analysis

Contamination across Different Time Periods of the Day

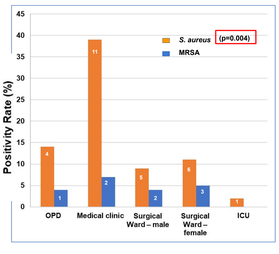
	Start of the day n(%) (n=30)	Mid of the day n(%) (n=30)	p value
CoNS	27(90.00)	28(93.33)	1.000
CoNS - Oxacillin MIC ≥ 4 µg/ml	26(86.67)	28(93.33)	0.671
S. aureus	5(16.67)	7(23.33)	0.519
MRSA	1(3.33)	2(6.67)	1.000

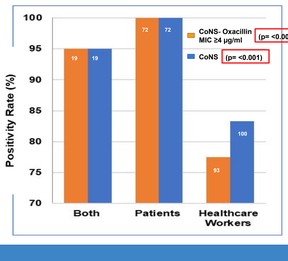
Contamination across Gender of Occupants

	Female n(%) (n=56)	Male n(%) (n=56)	p value
CoNS	49(87.50)	50(89.29)	0.768
CoNS - Oxacillin MIC ≥ 4 µg/ml	48(85.71)	49(87.50)	0.781
S. aureus	6(10.71)	5(8.93)	0.751
MRSA	3(5.36)	2(3.57)	1.000

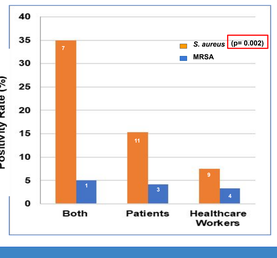


Contamination across different hospital units





Contamination by contact groups



Conclusions

- A **predominant and persistent CoNS** and a **moderate but persistent *S. aureus* and MRSA** contamination frequencies on high touch surfaces
- **Extremely high frequencies of CoNS with elevated oxacillin MIC** on high touch surfaces, maintaining **reservoirs of antimicrobial resistance genes** which facilitate horizontal gene transfer risk
- Statistically **significant different contamination frequencies** of Staphylococci across the **different hospital units** as well as **individual contact groups** interacting with the surfaces
- **High** rates of **phenotypically resistant but *mecA*-negative MRSA** strains - circulation of staphylococci with alternative resistant mechanisms

Future Research

- A study on the **dynamics of *SCCmec* transfer between the environmental CoNS** reservoirs and **clinical *S. aureus* isolates** within the hospital setting
- A follow-up study **employing Whole Genome Sequencing –**
  1. For the definitive characterization of the genetic mechanisms of resistance in environmental Staphylococci isolates
  2. To establish transmission pathways within the hospital.
- **A prospective, controlled interventional trial study and a multi-centre study–** to rigorously compare the efficacy of the current cleaning protocol

Key References

- Nkuwi, E.J. *et al.* (2018). Methicillin-resistant Staphylococcus aureus contamination and distribution in patient's care environment at Muhimbili National Hospital, Dar es Salaam-Tanzania. *BMC Research Notes*, **11(1):** [online]. Available at: https://doi.org/10.1186/s13104-018-3602-4 (Accessed:25 March 2024
- Institute for Health Metrics and Evaluation (IHME) of the University of Washington (2023). "The burden of antimicrobial resistance (AMR) in Sri Lanka" Available online: https://www.healthdata.org/sites/default/files/2023-09/Sri_Lanka.pdf (Accessed: 28 August 2025)
- Munasinghe, T. *et al.* (2021). Colonization with selected antibiotic resistant bacteria among a cohort of Sri Lankan university students. *BMC Infectious Diseases*, **21(1):** (onilne). Available at: https://doi.org/10.1186/s12879-021-06289-z (Accessed: 10 April 2024).

Contact
veranja.liyanapathirana@med.pdn.ac.lk
abhishekaperera99@gmail.com

Acknowledgement
- Funding by Alliance of International Science Organization (ANSO)