Figure 2: Patients with repeated screening for S. marcesens who were postive in at least one screening point

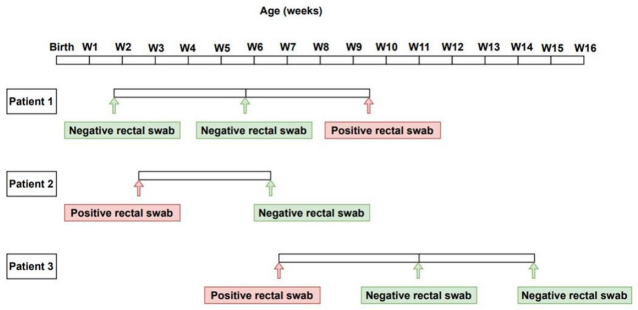
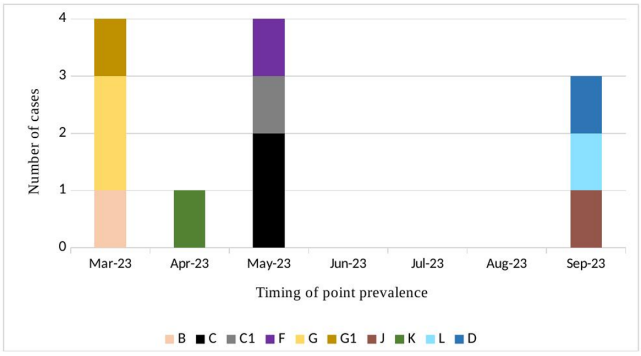


Figure 3: *S. marcesens* cases over 7 months of active surveillance



bed unit with all private rooms. Monthly point prevalence assessments by rectal screening were performed, alongside active surveillance for clinical infections associated with S. marcesens. Isolates from screening or clinical samples underwent assessment for relatedness using pulse field gel electrophoresis (PFGE). **Results:** Over the 7 month study period, 12 different patients (5.4%) were colonized/infected with S. marcescens. Among these, 10 patients (4.5%) were identified through rectal screening (316 rectal swabs were collected from 224 patients) and two patients (0.9%) exhibited positive clinical specimens (urine and endotracheal aspirate) in association with pyelonephritis and ventilator-associated pneumonia, respectively. Of the two clinical cases, one case showed a negative preceding rectal swab and the other detected through a clinical sample before the point prevalence date. The age at which a positive S. marcescens swab or positive clinical specimen was identified ranged from 4 to 66 days (median=18 days, IQR 5-38.8) (Figure 1). Sixty seven infants had repeated screening. Three out of 67 (4.5%) were colonized with S. marcescens, the timing and sequence of positive and negative testing are presented in Figure 2. Females demonstrated a higher positivity rate compared to males [9.1% (9/99) vs 2.4% (3/125), p=0.04, respectively]. PFGE analysis of all 12 (100%) isolates revealed a polyclonal pattern. Most cases were detected from March to May in 9/12 cases (75%). Ten different strains were identified. Notably, two strains demonstrated clusters of two cases each, one during March and the other during May (Figure 3). No mortality was reported among the cases. **Conclusions:** The study highlights the polyclonal nature of S. marcesens and raises questions about the utility of point prevalence in anticipating clinical cases or patient-to-patient transmission, especially in patients with clinical infection where there were no preceding positive screening tests.

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Comparative Analysis of Healthcare-associated Bloodstream Infections & CLABSI Surveillance in A Singaporean tertiary Hospital

Shalvi Arora, Singapore General Hospital; Sheena Jin Min Ong, Singapore General Hospital; Pinhong Jin, Singapore General Hospital; Aung Myat Oo, Singapore General Hospital; May Kyawt Aung, Singapore General Hospital; Yak Weng Darius Chan, Singapore General Hospital; Yang Yong, Singapore General Hospital; Ian Wee Liang En, Singapore General Hospital; Xiang Ying Jean Sim, Singhealth; Lai Chee Lee, Singapore General Hospital; Moi Lin Ling, Singapore General Hospital and Indumathi Venkatachalam, Singapore General Hospital

Background: Healthcare-associated central line associated bloodstream infection (HA-CLABSI) surveillance is important for monitoring healthcare-associated infections (HAIs) and evaluating effectiveness of infection prevention (IP) measures. However, implementing it is a laborious and time-consuming approach. Exclusive focus on central lines neglects HAI risk due to peripheral vascular catheters. This study aimed to assess whether HA-CLABSI incidence could be inferred from HA-bloodstream infection (BSI) trends and explore shift to HA-BSI surveillance. **Methods:** The study was performed in a Singaporean tertiary care hospital. Electronic medical records review was performed to determine whether positive blood cultures met Centers for Disease Control/National Health Safety Network (CDC/NHSN) definitions for HA-CLABSI and HA-BSI. Incident episodes of HA-BSI were included (excluding positive cultures repeated within 14 days). Incident organisms were explored to identify common causative pathogens (excluding same organisms isolated from cultures repeated within 14 days). CLABSI and BSI occurring ≥72hrs after admission were considered healthcare-associated. Patients under oncology or hematology service were considered immunocompromised. Incidence rates (IR) per 10,000 patient-days, patient characteristics and causative pathogens were compared between both indicators. **Results:** From January 2022 to October 2023, mean IR for HA-CLABSI was 0.63 (n=68) and for HA-BSI was 10.06 (n=1094). Median age of patients with HA-CLABSI was 66 years and HA-BSI was 68 years. HA-CLABSI and HA-BSI were more common in males (60.86% & 58.68%). Median duration

Figure: Incidence rate of HA-CLABSI from Jan 2022 to Oct 2023

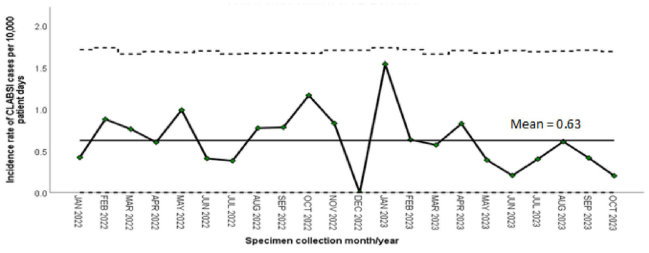

Figure: Incidence rate of HA-BSI from Jan 2022 to Oct 2023

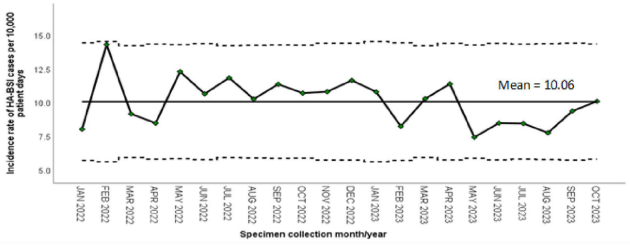

Table: Clinical characteristics of patients

Clinical characteristics	HA-CLABSI surveillance n=68 (%)	HA-BSI surveillance n=1094 (%)
Age (median, years)	66	68
Males	42 (61.8)	642 (58.7)
Immunocompromised	18(26.5)	359 (32.8)
Neonates	0 (0)	3 (0.27)
Burn injury	0 (0)	10 (0.9)
Length of hospital stay from admission to culture date (median, days)	19.5	12

Table: Breakdown by organisms:

Organism breakdown	HA-CLABSI surveillance n=84 (%)	HA-BSI surveillance n=1435 (%)
Gram negative bacteria	34 (40)	821 (57.2)
Enterobacterales	12 (14.3)	552 (38.5)
- *Escherichia coli*	1 (1.2)	221 (15.4)
- *Klebsiella pneumoniae*	5 (5.9)	182 (12.7)
- *Enterobacter cloacae*	1 (1.2)	47 (3.3)
- *Serratia marcescens*	5 (5.9)	30 (2.1)
- Other *Enterobactereacae* spp.	0 (0)	72 (5.0)
Pseudomonas spp.	7 (8.2)	96 (6.7)
Stenotrophomonas maltophilia	7 (8.2)	43 (3.0)
Acinetobacter baumannii/calcoaaceticeus complex	4 (4.7)	29 (2.0)
Other Gram negative bacteria	0 (0)	101 (7.0)
Gram positive bacteria	20 (23.8)	319 (22.2)
Staphylococcus spp.	15 (17.9)	157 (10.9)
Enterococcus spp.	4 (4.7)	99 (6.9)
Streptococcus spp.	0 (0)	11 (0.8)
Clostridium spp.	0 (0)	7 (0.5)
Other gram positive	1 (1.2)	45 (3.1)
Multi-drug resistant organisms	18 (21.2)	121 (8.4)
Enterococcus faecium (VRE)	9 (10.6)	53 (3.7)
Methicillin-resistant *Staphylococcus aureus* (MRSA)	9 (10.6)	44 (3.0)
Carbapenemase-producing CRE	0 (0)	24 (1.7)
- *Klebsiella pneumoniae*	0 (0)	11 (0.8)
- *Escherichia coli*	0 (0)	10 (0.7)
- *Enterobacter cloacae*	0 (0)	2 (0.1)
- *Klebsiella aerogenes*	0 (0)	1 (0.1)
Fungi	12 (14.1)	168 (11.7)
Candida spp.	12 (14.1)	150 (10.5)
Trichosporon spp.	0 (0)	6 (0.4)
Cryptococcus spp.	0 (0)	2 (0.1)
Fusarium solani complex	0 (0)	2 (0.1)
Other fungi	0 (0)	8 (0.6)
Mycobacterial spp.	0 (0)	6 (0.4)

between admission to HA-CLABSI was 20 days and to HA-BSI was 12 days. Median duration between central line insertion to HA-CLABSI was 16 days. Of 1094, 631 (57.7%) patients had vascular catheter(s) (i.e., IV cannula, port-a-cath, peripherally-inserted central catheter or central line) inserted at time of HA-BSI diagnosis, of whom 46 (7.3%) patients had CLABSI ±2days from positive blood culture. There was no significant correlation between monthly aggregate data from these indicators (Spearman's correlation coefficient= 0.36, p-value=0.1). Predominant organisms causing HA-CLABSI and HA-BSI were gram negative bacteria (GNB, 40% & 57.21%), gram positive bacteria (24.71% & 22.23%), and fungi. Common GNB in CLABSI patients were Pseudomonas spp. and Stenotrophomonas maltophilia (8.24%), followed by Serratia marcescens and Klebsiella pneumoniae (5.88%). The frequent GNB in HA-BSI patients were Escherichia coli (15.4%), Klebsiella pneumonia (12.68%), and Pseudomonas spp. (6.69%). Common multi-drug resistant organisms were vancomycin-resistant Enterococcus faecium (10.59% & 3.69%) and methicillin-resistant Staphylococcus aureus (10.59% & 3.07%). **Conclusion:** HA-BSI did not correlate with HA-CLABSI. HA-BSI reflects heterogenous population outcomes. For utilization as surveillance indicator, further assessment on exclusion criteria is required to improve specificity.

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epiXact-ONT: Long-read whole genome sequencing for rapid outbreak detection and comprehensive plasmid transmission analysis

Emma Briars, Day Zero Diagnostics; Ian Herriott, Day Zero Diagnostics; Allison Brookhart, Day Zero Diagnostics; Talia Hollowell, Day Zero Diagnostics; Nicole Billings, Day Zero Diagnostics; Miriam Huntley, Day Zero Diagnostics and Mohamad Sater, Day Zero Diagnostics

Background: Healthcare associated infections (HAIs) are a major contributor to patient morbidity and mortality. HAIs are increasingly important due to the rise of multidrug resistant pathogens which can lead to deadly nosocomial outbreaks. Traditional methods for investigating transmissions are slow, costly and have poor detection resolution. In addition, plasmid transmission which can horizontally transfer critical resistance and virulence genes is not part of routine infection control practice due to lack of comprehensive and cost-effective methods capable of identifying both pathogen and/or plasmid transmission. Here we demonstrate the utility of the Oxford Nanopore Technologies (ONT) platform for whole genome sequencing (WGS) based pathogen and plasmids transmission analysis. **Methods:** We developed a rapid end-to-end process that includes sample preparation, sequencing optimized for generating long-reads and bioinformatics workflow customized for error-prone ONT data. We use Flye to generate de novo assemblies and a secondary bioinformatics step to identify each circular sequence. Individual circular sequences with an Ori (≥1) are identified. For pathogen clonality analysis we perform a pairwise mapping-based chromosomal sequences comparison eliminating need for an external reference genome. Similarly, individual plasmids are separated and compared pairwise. We annotate both the circularized chromosomal and plasmid sequences for known resistance and virulence genes. **Results:** We performed ONT (and confirmatory Illumina) sequencing of the genomes of 20 bacterial isolates originating from 5 HAI investigations previously performed at Day Zero Diagnostics using epiXact®, our Illumina-based HAI sequencing and analysis lab service. ONT-based clonality determination had 100% agreement with the Illumina based pipeline. We also found that using the outbreak-specific assembled genomes instead of an external reference increased the SNP-calling resolution in the ONT pipeline. We also identified sets of clonal isolates with both identical plasmids and distinct plasmids; as well as sets of non-clonal isolates with identical plasmids and distinct plasmids. In one subset of 7 multi-species isolates, we identified 2-7 circularized plasmid sequences in each isolate, all harboring known resistance genes. 4 plasmids were found in multiple isolates, with each plasmid appearing in between 2 and 4 distinct isolates. Notably, blaNDM was identified in at least 1 plasmid in each isolate. **Conclusion:** We demonstrate the utility of ONT for comprehensive HAI investigations, establishing the potential to transform healthcare epidemiology with rapid outbreak determination covering pathogen and plasmid transmission in < 2 4 hours from sample receipt.

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