Next Generation Sequencing in a Hospital Outbreak Investigation– Experience from the Developing World

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Background: Use of Next generation sequencing (NGS) technology when compared to conventional phenotypic typing methods in hospital outbreak analysis facilitates not only rapid & accurate identification of the pathogen but also identifies the transmission patterns of the pathogen and thereby helps rapid control of an outbreak. Here we describe our experience with NGS in the understanding of a hospital outbreak due to *Serratia marcescens* blood stream infections.

Methods: A sudden increase of *Serratia marcescens* blood stream infections predominately from patients in cardiac care unit (CCU) ,intensive care units (both adult (AICU) & neonatal (NICU)) occurred in a tertiary care hospital in South India between November 2014 and April 2015. Initially, using conventional phenotypic methods, clinical & environmental sampling, pathogen identification and sensitivity (Vitek Compact II) was performed. Then a systematic outbreak approach using NGS technology with Nextera XT chemistry (Illumina Inc, San Diego, CA, USA) for a 250-bp paired-end sequencing run on an Illumina MiSeq sequencer from stored isolates was performed to understand the outbreak.

Results: During study period 12 blood stream isolates from patients & 4 environmental isolates of *Serratia marcescens* were identified in 3 different time periods as mentioned in table1 below.

Table-1 Source, Phenotypic & NGS pattern of *Serratia marcescens* bacterial isolates

Month & year	No of isolates	Source of Isolate	Phenotypic pattern	NGS pattern	Conclusion
Period 1: (outbreak) November 2014 – January 2015	4	CCU (All 4 Clinical isolates)	**Pattern 1** (all antibiotics sensitive)	**Cluster 2**	Outbreak, both phenotypic methods & NGS identified. Source not identified
Period 2 (non-outbreak) February 2015- March 2015	4	2 isolates from NICU & 2 isolates from AICU(all 4 Clinical isolates)	**Pattern 1** (all antibiotics sensitive)	**Unique pattern**	Non-outbreak Phenotypic pattern suggested outbreak but NGS patterns were unique
Period 3 (outbreak) April 2015	8	AICU Clinical 4 & Environmental 4)	**Pattern 2** (6 isolates were amp C +ESBL positive of which 4 were clinical isolates and 2 were environmental isolates from the heparin solution used for patients CVP line) Different susceptibility patterns seen in 2 environmental isolate from the noradrenaline solution)	**Cluster 1** – 8 isolates (4 clinical & 4 environmental) All isolates belonged to the same NGS pattern though 2 environmental isolates differed in antibiogram	Outbreak identified by both phenotypic typing & NGS in 6/8 isolates. 2 environmental isolates of the same outbreak identified only through NGS (differed in antibiogram).

Phenotypic pattern of all 8 *Serratia* isolates identified till March 2015 were similar, but NGS showed 4 isolates in Period 1 (November to January) belonged to one cluster and the remaining were unique (not part of the outbreak). However, during April 2015 phenotypic methods identified 6/8 isolates as belonging to 1 pattern but NGS correctly identified all 8 isolates as belonging to the same outbreak cluster.

Conclusion: Compared to conventional phenotypic typing methods, NGS technology clustered bacterial isolates clearly, tracked the path of the outbreak (CCU&AICU) and thereby helped the investigators to identify the source & develop control measures for the outbreak in our center.

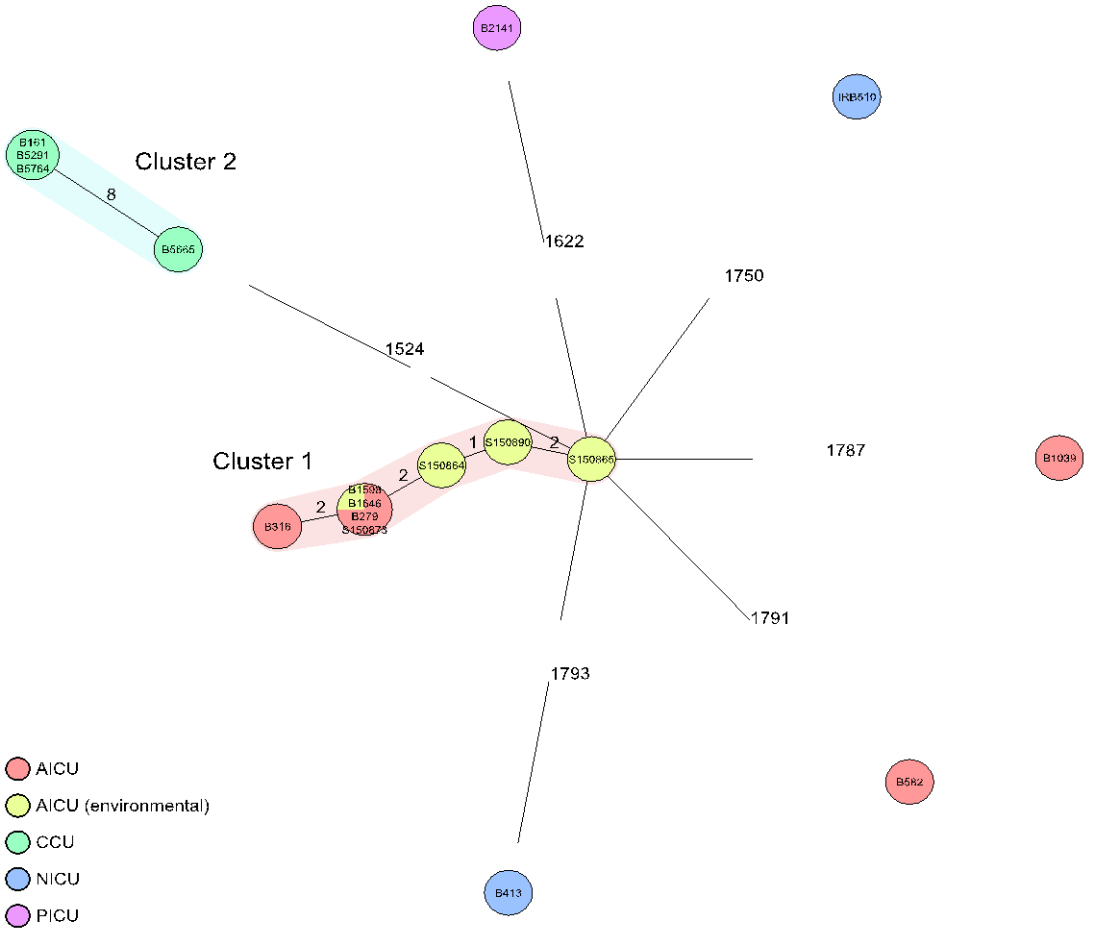

Figure 1: Minimum spanning tree based on cgMLST allelic profiles of 17 *S. marcescens* isolates, pairwise ignoring missing values. Each circle represents an allelic profile based on sequence analysis of 2,780 cgMLST target genes. The numbers on the connecting lines illustrate the numbers of target genes with different alleles. Isolates belonging to the same cluster are shaded.