

# Comparative Genomics of Serial Isolates: Can Genome Mapping Provide a Road Map for Clinicians?

PE Waterman<sup>1,3</sup>, MC Riley<sup>1</sup>, YI Kwak<sup>1</sup>, P McGann<sup>1</sup>, A Summers<sup>1</sup>, X Lin<sup>2</sup>, R Clifford<sup>1</sup>, J Hang<sup>2</sup>, and EP Lesho<sup>1,3</sup>

<sup>1</sup> Multidrug-resistant organism Repository and Surveillance Network, <sup>2</sup> Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD

<sup>3</sup> Uniformed Services University of the Health Sciences, Bethesda, MD



## Abstract

**Background:** Optical genome mapping (OGM) involves the immobilization of DNA molecules on a substrate, digestion with a restriction enzyme, and assembly into a full genome restriction map. The DoD is a leading consumer of this technology, albeit slow, expensive, and unproven clinically. One setting of use may be in the polytrauma war wounded patient with multiple bacterial isolates. Current lab standard of care is to refer subsequent like-appearing positive cultures to the initial culture. This approach can miss important organism changes with therapeutic or morbidity and mortality consequences.

**Methods:** Multidrug-resistant Gram-negative bacteria or methicillin-resistant *S. aureus* serially isolated from the same patient along the evacuation chain from Afghanistan to the U.S., with concern for breakdown in infection control, underwent identification and susceptibility testing followed by pulsed-field electrophoresis (PFGE). OGM was then performed to see if PFGE missed important differences in strain typing or clinically relevant genomic changes.

**Results:** In all infection control settings, OGM and PFGE strain relatedness was concordant. In settings involving serial isolates spanning 50 days, PFGE revealed 3 predominant clones. Two patients shared 2 clones, across 3 hospitals. OGM of these isolates did not identify unique differences between the individual isolates. However, initial OGM did identify a 90 kb insertion in a patient with numerous isolates over a 50 day period, not revealed by PFGE. The insertion was coincident with a change in phenotype that occurred over 24hrs.

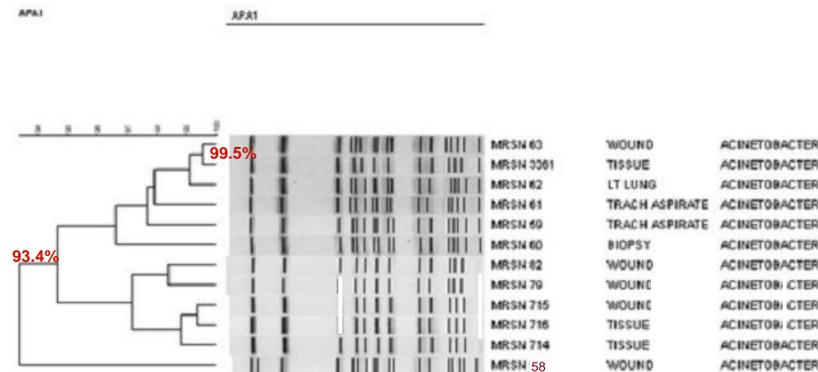
**Conclusions:** Despite higher cost and resolution, OGM did not provide more information than PFGE for infection control. OGM revealed potential mechanisms for rapid evolution of drug resistance in the same host. If larger studies replicate this finding, the practice of referring serial isolates from the same patient may need to be reconsidered.

## Clinical Data

ISOLATE #	SOURCE/DPI	LOCATION	AST
MRSN 714	Wound/day 3	OCONUS #2	SENS: Amik, Tobra, Col INTERMED: Levo
MRSN 715	Wound/day 3	SAME	SENS: Amik, Tobra, Col INTERMED: Levo
MRSN 716	Wound/day 3	SAME	SENS: Amik, Tobra, Col
MRSN 58	Wound (hip)/day 12	CONUS #1	SENS: Amik INTERMED: Levo
MRSN 59	Respiratory/day 13	SAME	SENS: Amik, Tobra INTERMED: Levo
MRSN 60	Tissue (sacral)/day 13	SAME	INTERMED: Amik, Tobra
MRSN 61	Respiratory/day 15	SAME	SENS: Amik, INTERMED: Tobra
MRSN 62	Lung (BAL)/day 16	SAME	SENS: Amik INTERMED: Tobra
MRSN 63	Tissue (pelvis)/day 17	SAME	SENS: Amik INTERMED: Tobra
MRSN 79	Wound (abdomen)/day 50	SAME	SENS: Amik INTERMED: Tobra
MRSN 82	Wound (abdomen)/day 53	SAME	SENS: Amik, Tobra

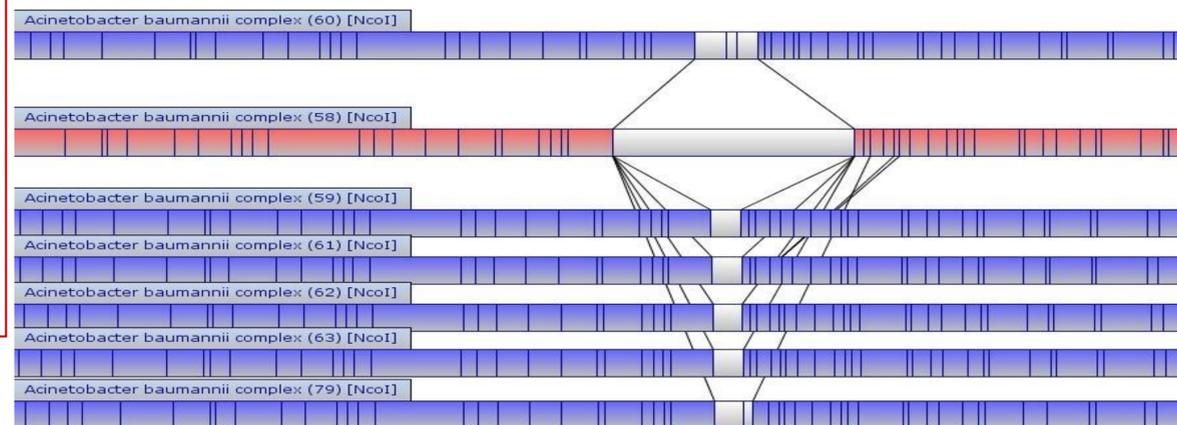
**Table 1.** DPI = Day post injury; Cx + = culture positive; AST = antimicrobial sensitivity per Vitek/Phoenix/Colistin Kirby-Bauer @; CONUS = continental United States (#1 = Walter Reed); OCONUS #2 = Outside Continental United States (Landstuhl, Germany); Amik = amikacin; Levo = levofloxacin; Tobra = tobramycin; Col = Colistin; SENS = sensitive; INTERMED = intermediate

## PFGE Data

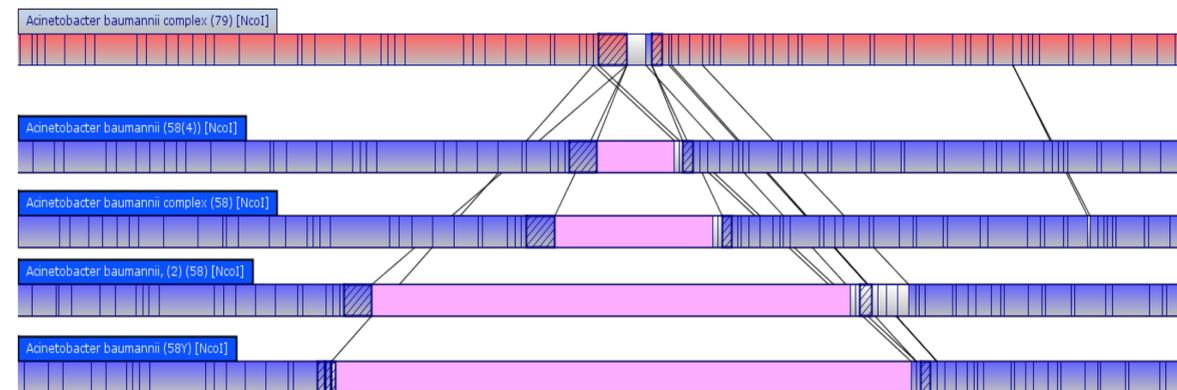


**Figure 1.** PFGE of serial isolates. MRSN 63 & MRSN 3361 (99.5%); MRSN 58 & all else 93.4% correlated

## Optical Mapping Data



**Figure 2.** Optical maps of series of isolates highlighting large area of difference in MRSN 58.



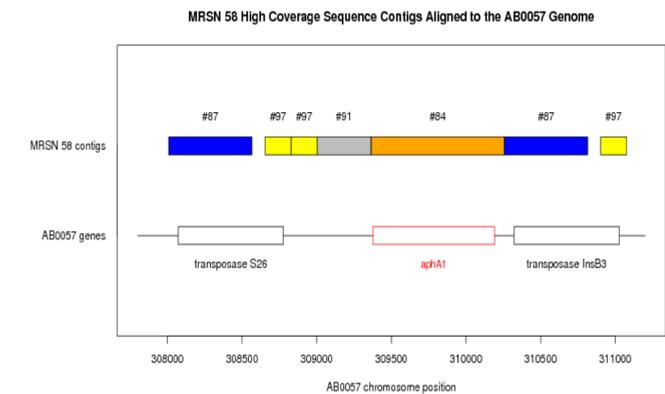
**Figure 3.** Whole genome alignment of ACB 79 and 4 mapped "58s". The insertion in MRSN 58 varies from 43kb to greater than 272 kb. Matching ends highlighted; region of interest in pink. White fragments are unaligned, blue are aligned in agreement, red means ≥ one sequence aligns in agreement.

## Acknowledgements & References

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Menard R, Molinas C, Arthur M, Duval J, Courvalin P, Leclercq R. Overproduction of 3'-aminoglycoside phosphotransferase type 1 confers resistance to tobramycin in Escherichia coli. AAC. 1993. Jan;37(1):78-83/

## 454 Pyrosequencing



**Figure 4.** *de novo* assembled sequence contigs from *Acinetobacter baumannii* strain MRSN 58 mapped onto the reference strain AB0057 genome sequence. DNA sequences were aligned using BLAST. The four sequence contigs have greater than 50-fold higher read coverage compared to the mean for the entire genome. Contigs #91 and #84 align to unique locations in the reference genome; contigs #87 and #97, homologous to transposons, align to multiple locations in the reference genome.

## Conclusions

- The drug resistance gene *aphA1*, associated with resistance to kanamycin and tobramycin is marked in red.
- Elevated read coverage of the *aphA1* region suggests that this DNA sequence is present at high copy number in MRSN 58. This region shows normal read coverage in the related strains MRSN 60 and 79, which have different drug susceptibility profiles than MRSN 58.
- Optical genome mapping reveals that MRSN 58 isolates have a variable size insertion relative to MRSN 60 and 79.
- We have used quantitative PCR to verify that this insertion contains multiple copies of the *aphA1* region.