

# Comparison of MALDI-TOF Mass Spectra with Variable Nucleotide Tandem Repeats profiles (VNTR) of *Pseudomonas aeruginosa* in an outbreak scenario

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## Introduction

*Pseudomonas aeruginosa* (PAER) is one of the more common nosocomial pathogens and is an increasing cause of outbreaks on augmented care units. It is frequently found in water sources both inside and outside hospitals and has been implicated with increased morbidity and mortality. During outbreak scenarios PAER isolates require typing to confirm similarity. The current typing method in the UK in the UK reference laboratory is nine locus Variable Number Tandem repeat (VNTR) analysis, a skilled specialist method taking at least a day to perform. This results in a time delay in results receipt and crucial information to the infection control team. Matrix Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) is becoming more widespread in microbiology laboratories as a method of identification. Spectra can be achieved within minutes and would provide a quick way to confirm outbreak isolates. Here we compare cluster analysis of MALDI-TOF MS spectra with VNTR types.

Table 1: Sample sites of *P. aeruginosa* isolates

VNTR Profile	Blood	ET secretions	Eye	Grain	Nasal screen	NHL	Nephrology swab	NPA	Rectal screen	Rectal swab	Sputum	Urine	Wound
10,3,2,4,2,1,6,6,9					1								
11,3,5,5,3,4,9,2,13	2												
11,4,5,2,2,2,8,2,12	2				2	1			1				
11,4,5,5,4,3,8,1,8	3	3	3	1	5			1			1		
11,6,2,2,1,3,8,2,12	1								8	1		2	1
12,2,1,-,5,2,4,5,9	1	1											
12,5,-,2,6,1,6,4,12	1				1		1						

Table 2: Antibigram profiles of *P. aeruginosa* isolates

No	Specimen type	Gen	Tob	Ani	Col	Cip	Caz	Imi	Mer	Pip/Taz	VNTR Profile
38	Blood	S	S	S	S	S	S	S	S	S	11,4,5,5,4,3,8,1,8
2	Grain	S	S	S	1	R	R	R	R	R	11,4,5,5,4,3,8,1,8
1	Eye	S	S	S	S	S	S	S	S	S	11,4,5,5,4,3,8,1,8
40	Blood	S	S	S	S	S	S	S	S	S	11,4,5,5,4,3,8,1,8
45	Nasal screen	S	S	S	S	S	S	S	S	S	11,4,5,5,4,3,8,1,8
37	Blood	S	S	S	S	R	R	1	1	1	11,5,4,5,4,2,-,6,7
11	Blood	S	S	S	R	R	S	S	S	S	11,3,5,5,3,4,3,2,13
16	Nasal screen	S	S	S	S	S	S	S	S	S	11,3,5,5,3,4,3,2,13
8	Urine	S	S	S	S	S	S	S	S	S	11,6,2,2,1,3,8,2,8
10	ET secretions	S	S	S	S	S	S	S	S	S	11,6,2,2,1,3,8,2,17
12	Nephrology swab	S	S	S	S	S	S	S	S	S	12,5,-,2,6,1,6,4,9
28	lblood	S	S	S	S	S	S	S	S	S	12,5,-,2,6,1,6,4,9
18	NPA	S	S	S	S	R	S	S	S	S	12,5,-,2,6,1,6,4,13
33	blood	S	S	S	S	S	S	S	S	S	11,4,5,2,2,1,8,2,-
47	Blood	S	S	S	S	S	S	S	S	S	12,2,1,-,5,2,4,5,13
29	ET secretions	S	S	S	S	S	S	S	S	S	12,2,1,-,5,2,4,5,9

## Methods

VNTR typing was performed at the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit on 47 PAER clinical or screening isolates from patients (Table 1). All isolates were from patients on Neonatal unit (NNU) during outbreak surveillance. PAER was subsequently isolated from both water, clinical and screening samples and susceptibility testing performed by disc diffusion. In addition, all PAER were extracted using Manufacturers' standard extraction method and MS analysis performed using FlexAnalysis and BioTyper 3.0 (Bruker Daltonik GmbH). Hierarchical analysis on the spectra by the Euclidean distance was performed using the PCA dendrogram tool. VNTR types and spectra dendrograms were compared for correlation. A similarity cut off of 4 was chosen arbitrarily for the MALDI group definition.

Table 3. Numbers of *P. aeruginosa* isolates in VNTR profile and MS groups

VNTR profile	No. of isolates in VNTR group	MS GROUP	Numbers of isolates in MS group
11,4,5,5,4,3,8,1,8	17	Gp 1	15
		Gp 2	2
11,5,4,3,4,2,-,6,7	1	Gp 1	1
11,3,5,5,3,4,3,2,13	3	Gp 2	2
		Gp 2	1
11,6,2,2,1,3,8,2,W	12	Gp 2	12
11,6,2,2,1,3,7,2,10	1	Gp 2	1
11,4,5,2,2,1,8,2,X	6	Gp 1	1
		Gp 2	5
10,3,2,4,2,1,6,6,9	1	Gp 2	1
12,2,1,-,5,2,4,5,Y	2	Gp 2	1
		Gp 3	1
12,5,3,2,6,1,6,4,Z	4	Gp 1	2
		Gp 2	1
		Gp 3	1

W=8, 12 or 17, X=7, 8 or 12, Y=9 or 13, Z=9, 12 or 14

## Results

MALDI-TOF MS spectra clustered into 3 main groups compared with 9 main VNTR groups (Figure 1). Within the 9 groups, VNTR showed 16 distinct subpopulations based upon differences at the final locus. The MS spectra clustered the majority of the 9 main VNTR types together (Table 3), with 15/17

"11,4,5,5,4,3,8,1,8" VNTR types in MS group 1 and 12/12 "11,6,2,2,1,3,8,2,W" clustering in MS group 2.

Two samples most similar according to MALDI-TOF spectra were samples 5 and 7. These were both recovered from eye swabs on separate patients on NNU within days of each other. However in another instance, spectra of two isolates (30 & 46) clustered in group 2 compared with most closely linked VNTR types in group 3.

## Conclusions

Results suggest better discrimination when using VNTR when compared with MALDI-TOF MS. However MS may provide useful infection control information within hours of PAER isolation, although there is the caveat that the results may be unreliable and potentially misleading. This warrants further study using a larger number of isolates.

Figure 1: MS spectra dendrogram of *P. aeruginosa* isolates

