Pseudomonas aeruginosa PcrV and Psl Antigens are Conserved in Diverse Hospital Isolates Collected from an International Surveillance Study

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Background

Ventilator-associated pneumonia due to *Pseudomonas aeruginosa* (PA) is associated with high rates of mortality, morbidity, increased intensive care unit length of stay and substantial economic burden. MEDI3902 is a bivalent, bispecific human IgG1κ mAb that selectively binds to both the type 3 secretion (T3S) injectisome protein PcrV and Psl exopolysaccharide on the surface of PA and is being developed for the prevention of nosocomial pneumonia caused by PA. MEDI3902 binding to PcrV prevents T3S injectisome-mediated host cell cytotoxicity, while binding to Psl mediates opsonophagocytic killing of PA by host phagocytic cells and may also inhibit attachment of the bacterium to host epithelial cells

Methods

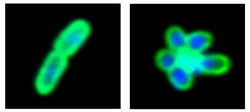
To better understand the prevalence of the *pcrV* gene and *psl* operon (expression of Psl is mediated via a 20 KB operon) in *P. aeruginosa* clinical isolates, we performed whole genome sequencing on 913 isolates collected from diverse patient populations and geographical locations in 43 countries from 2004-2014. Whole genome sequence data was analyzed for the presence of the *pcrV* gene and *psl* operon (*pslA* to *pslO*). The predicted amino acid sequence changes in the PcrV protein were compared to PA reference strain PAO1. Psl expression from select isolates lacking one or more genes from the *psl* operon was evaluated by an anti-Psl ELISA assay.

Results

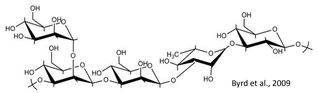
Analysis showed that the *pcrV* and *psl* genes were present in 99% and 96% of the isolates, respectively. PcrV amino acid sequence variation revealed 46 clusters/genotypes. A naturally occurring set of isolates that are null in one or more *psL* genes was uncovered. In addition, a set of PA7-like isolates with wide temporal and global distribution were identified.

Introduction

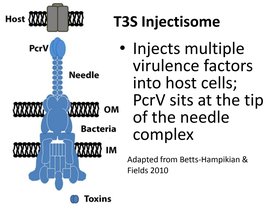
- We recently described mAbs targeting the *P. aeruginosa* T3SS protein PcrV (Warrener et al., 2014) and Psl EPS (DiGiandomenico et al., 2012), which are components of bispecific clinical candidate MEDI3902 (DiGiandomenico et al., 2014).
- Psl EPS is an abundant, serotype independent surface sugar polymer that is important in tissue colonization, biofilm formation (persistence) and immune evasion.
- PcrV forms the tip of the T3SS which can inject exotoxins into host target cells.



Confocal microscopy images of *P. aeruginosa* stained with a Alexa Fluor 488 labelled anti-Psl mAb



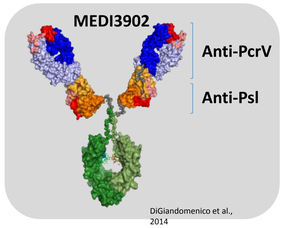
Psl EPS individual subunit



T3S Injectisome

- Injects multiple virulence factors into host cells; PcrV sits at the tip of the needle complex

Adapted from Betts-Hampikian & Fields 2010



- MEDI3902 prevents *P. aeruginosa* cytotoxicity (anti-PcrV) and promotes Psl mediated opsonophagocytic killing activity (anti-Psl)

Figure 1: *P. aeruginosa* Psl exopolysaccharide and the T3SS injectisome

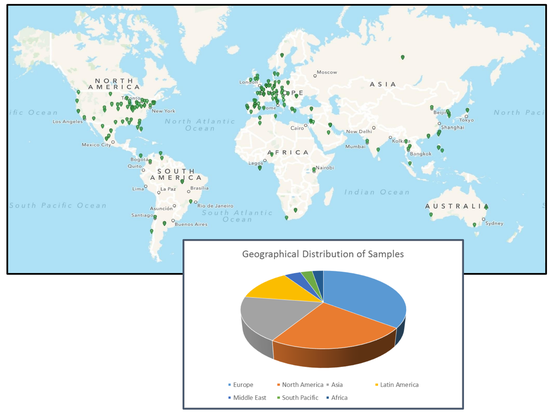


Figure 2: Map of *P. aeruginosa* isolates collection sites and plot of geographical distribution.

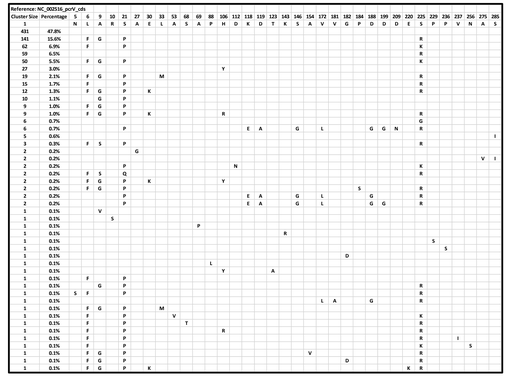


Table 1: The sequence of the *pcrV* gene was determined by whole genome sequencing. The consensus DNA sequences were translated to PcrV protein sequences, and amino acid variants were noted based on comparison to the published PAO1 sequence.

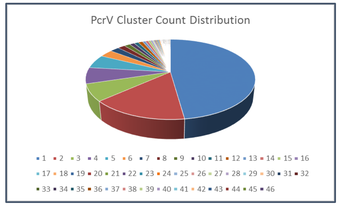


Figure 3: PcrV Cluster Count Distribution.

					MLST Alleles							T3SS Genes					T2SS Genes			
Sample	Region	Country	Year	ST	acs	aro	gua	mut	nuo	pps	trp	pcrV	exoS	exoT	exoU	exoY	PA7_1410_gspJ	PA7_1417_ompP	PA7_1419_ChpD	PA7_1420_regulator
1	Asia	India	2008	2211*	87	70	79	80	53	487	129*	-	-	-	-	-	PA7_1410_gspJ*?	PA7_1417_ompP*?	PA7_1419_ChpD*	PA7_1420_regulator*
2	Asia	China	2009	Nf*	87*	34	43	60	53	107*	196*	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
3	Europe	Germany	2012	2211	87	34	114	37	86	100	170	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
4	Asia	Thailand	2013	1978	45	148	15	35	17	8	177	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
5	Latin America	Mexico	2013	1978	45	148	15	35	17	8	177	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
6	South Pacific	Philippines	2014	1978	45	148	15	35	17	8	177	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
7	North America	United States	2013	1328	118	104	85	86	42	54	90	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*?	PA7_1419_ChpD*?	PA7_1420_regulator*?
8	Latin America	Mexico	2014	1225	118	106	85	92	60	54	72	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*?	PA7_1419_ChpD*?	PA7_1420_regulator*?
9	Middle East	Israel	2014	2211	87	34	114	37	86	100	170	-	-	-	-	-	PA7_1410_gspJ*	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
10	Asia	Japan	2014	Nf*	87	34*	43	60	53	108	144	-	-	-	-	-	PA7_1410_gspJ	PA7_1417_ompP*	PA7_1419_ChpD*	PA7_1420_regulator*
11	Asia	Taiwan	2014	1195	87	34	43	37	53	107	126	-	-	-	-	-	PA7_1410_gspJ	PA7_1417_ompP	PA7_1419_ChpD	PA7_1420_regulator

Figure 4: PA7-like isolates lacking T3SS elements but contain a T2SS as described by Cadoret *et al.* Gene presence is indicated by gene name and absence is indicated by a '-' in the table.

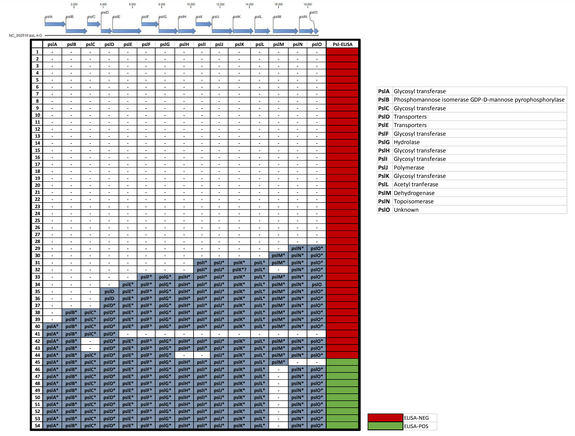


Table 2: The presence of the *psl* operon was determined by analysis of whole genome sequencing data. Gene presence is indicated by gene name and absence is indicated by a '-' in the table. Psl expression was determined by western blot.

Conclusions

- To our knowledge, this is the first international study to examine *pcrV* and *psl* gene conservation and diversity in hospital-associated isolates.
- Analysis showed that the *pcrV* and *psl* genes were present in 99% and 96% of the isolates, respectively.
- A naturally occurring set of isolates that are null in one or more *psL* genes was uncovered.
- 11 PA7-like isolates were identified with wide temporal and global distribution.
- PcrV and Psl are good targets for prophylactic mAb and vaccine development.

References:

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