Characteristics of a Multiple Drug Resistant Strain of *Pseudomonas spp.* Resulting from a Potentially Novel Gene Transfer of *Salmonella* Genomic Island 1.

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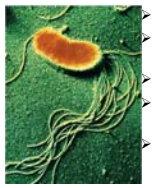
Abstract

Excessive use of antibiotics has applied evolutionary stress to the bacterial population, generating genes responsible for antibiotic resistance through random mutation and natural selection. Acquisition and accumulation of genes via horizontal gene transfer has led to resistance to multiple antibiotics, known as multi drug resistance (MDR). In this study, we reported the isolation and characterization of a MDR *Pseudomonas strain* D9. Sequencing of 16SrRNA yielded 99% sequence homology to *Pseudomonas fulva*. This isolate is resistant to 10 of 13 antibiotics tested using minimum inhibitory concentration (MIC) testing. Sequence analysis indicates that this strain harbors a variant of the MDR region of *Salmonella* genomic island 1 (SGI1). Shotgun,454, and PCR sequencings have yielded highly homologous (between 85-100%) fragments corresponding to an MDR region of *Salmonella* genomic island 1 (SGI1). This MDR region does not appear to be plasmid-borne and has not been associated with any *Pseudomonas* strains studied previously. The homology of the MDR regions between the this *Pseudomonas* strain and *Salmonella* SGI indicate a novel horizontal gene transfer between the two genera.

Introduction

- Excessive use of antibiotics has applied evolutionary stress to the bacterial population, generating genes responsible for antibiotic resistance through random mutation and natural selection.
- Acquisition of genes via horizontal gene transfer has led to resistance to multiple antibiotics, known as multi drug resistance (MDR).
- Recently, a MDR strain of *Pseudomonas sp.*, designated strain D9, exibiting resistance to a core group of antimicrobial agents including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (commonly abbreviated ACSSuT) in addition to gentamicin, kanamycin, amoxicilin, metronidazol, cephalosporin, penicillin, rifampicin, vancomycin.
- Normally, *Pseudomonas sp.* is an environmental bacterium not normally associated with multiple drug resistance.
- Preliminary sequence analysis indicates that this strain may harbor a variant of *Salmonella* genomic island 1 (SGI1)



  - SGI1 confers resistance to ACSSuT.
  - SGI1 is a 47-Kb fragment of *Salmonella enterica* Typhimurium genome, the most prevalent *Salmonella* serovar in the United States and Canada.
  - SGI1 has been shown to transfer horizontally via helper plasmids.
  - Resistance is clustered in a GC rich MDR region near the 3' end of SGI1 which contains genes which allow for horizontal gene transfer.
  - A high level of recombination occurs within this region of the genomic island. If SGI1 is present within our *Pseudomonas* isolate it will represent a novel gene transfer between these organisms.
- Previously we identified the bacterium using 16SrRNA sequencing as *Pseudomonas fulva*,
  - *P. fulva* is an environmental bacterium found in households.
  - *P. fulva* is associated with plant roots where it exhibits phytopathogenic fungi antagonism.
- Only two cases of infection by *P. Fulva* have been documented, one bloodstream infection and one case of meningitis, both exhibiting different antibiotic resistance.
- We investigated antibiotic minimum inhibitory concentration (MIC), and 454 whole genome sequencing.

Methods

MIC determination

Adjust inoculum to 5 x 10^5 cells in Mueller Hinton broth → Serial dilute antibiotics ranging from 0.25-512 µg/mL → Add resazurin growth indicator (6.75 g/L) → Incubate at 37° C for 24 hours

SGI1 plasmid testing

Extract plasmids using Promega Wizard and Nucleospin kits. → Digest with HindIII restriction enzyme. → Run product on 1.5% agarose for 10 minutes at 100V → Confirm results with *E. coli* transformation

SGI1 sequencing

Shotgun sequencing → BLAST alignment → Design primers to fill gaps and run PCR → Sequencing at City of Hope → BLAST alignment

Genomic DNA purification using Qiagen extraction kit → 4 5 4 sequencing at CSISP in Spain

Results

1. Identification of *Pseudomonas sp.* isolate.

Firgure 1: Sequencing of 16S ribosomal subunit yielded 99% homology to *Pseudomonas fulva* . Three mismatches were found as a result of sequencing errors yielding a nonspecific nucleotide at these points

2. *Pseudomonas* MDR sequencing and comparison to SGI1

A. Location of alignments

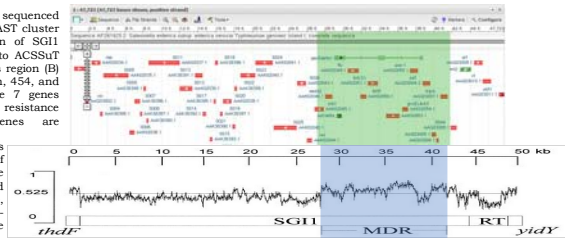
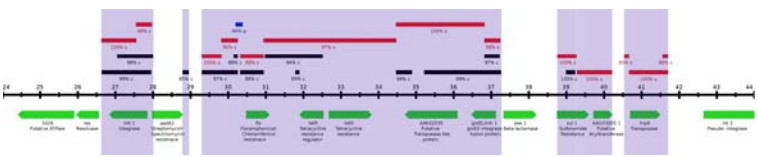


Figure 2: All significant sequenced fragments aligned with BLAST cluster within the GC rich region of SGI1 responsible for resistance to ACSSuT (A). A detailed view of this region (B) reveals that a after Shotgun, 454, and PCR sequencing, 5 of the 7 genes responsible for antibiotic resistance and resistance regulatory genes are within our sequenced regions. Sequencing has revealed fragments of *aadA2*, the gene responsible to Streptomycin and spectinomycin resistance, while *pse1*, a beta-lactamase, has yet to be found.

B: Sequence alignment against SGI1



C: Resistance comparison and gene homology

Antibiotic	MIC (µg/mL)	SGI1 MIC* (µg/mL)	Antibiotic breakpoint (µg/mL)	Resistance genes reported in SGI1	Sequence Homology (%)
Ampicillin	512	>10	>16	pse-1	-
Chloramphenicol	256	256	>32	Putative acyl transferase & flo	88/97 & 100
Streptomycin	32	>10 IU	>32	aadA2	98/85
Tetracycline	512	128	>16	tetR & tet(G)	97 & 97
Spectinomycin	4	>100	-	aadA2	98/85
Sulfamethoxazole	>512	>200	>38	sul1	100/-/100

Table 2: Comparison of MIC values and the MDR sequences between D9-A and *Salmonella*. The results show that chlormaphenicol resistance in D9-A matches SGI1 resistance values while spectinomycin is below the corresponding resistance level in SGI1. For all other antibiotics, D9-A is at or above the values conferred by SGI1. Our sequenced fragments show >85% homology for the genes responsible for ACSSuT resistance. No fragments containing *Pse1* gene have been found.

3. Antibiotic Minimum Inhibitory Concentration (MIC) testing

A. Antibiotic groups and MIC

Antibiotic	Antibiotic type/group	Mechanism	MIC* (µg/mL)	Antibiotic breakpoint (µg/mL)	Susceptable/ Resistant	Resistance genes reported in SGI1
Vancomycin	Glycopeptide	Inhibits cell wall synthesis.	213.3	none***	-	-
Cefotaxime	β-Lactam	Inhibits cell wall synthesis.	32	>2	resistant	-
Ampicillin	β-Lactam	Inhibits cell wall synthesis.	512	>16	resistant	yes
Chloramphenicol	Chloramphenicol	Binds to 50S ribosomal subunit, inhibiting protein synthesis.	256	>32	resistant	yes
Erythromycin	Macrolide	Binds to 50S ribosomal subunit, inhibiting protein synthesis.	32	>8	resistant	-
Gentamicin	Aminoglycoside	Binds to 30S ribosomal subunit, inhibiting protein synthesis.	16	>16	resistant	-
Kanamycin	Aminoglycoside	Binds to 30S ribosomal subunit, inhibiting protein synthesis.	128	>64	resistant	-
Streptomycin	Aminoglycoside	Binds to 30S ribosomal subunit, inhibiting protein synthesis.	32	>32	resistant	yes
Tetracycline	Polyketide	Binds to 30S ribosomal subunit, inhibiting protein synthesis.	512	>16	resistant	-
Spectinomycin	Aminocyclitol	Binds to 30S ribosomal subunit, inhibiting protein synthesis.	4	none**	-	yes
Sulfamethoxazole	Sulfonamide	Competetive analogue to para-aminobenzoic acid in folic acid synthesis.	>512	>38	resistant	yes
Rifampicin	Rifamycin	Binds to β-subinit of RNA polymerase, inhibiting transcription.	26.7	none***	-	-
Metronidazol	Nitromidazole	Metabolized to create toxic products, damaging DNA.	>512	>4	resistant	-

*Final minimum inhibitory concentration pending standardized organism control to test accuracy of dilution concentrations.
** No breakpoint has been established for *Pseudomonas* as spectinomycin is primarily used to treat infection by *Neisseria gonorrhoeae.*
*** No breakpoint is established as *Pseudomonas* is a poor target for this drug.

Table 1: Minimum Inhibitory Concentration results yielded 10 of the 13 antibiotics tested with MICs higher than the susceptibility/resistance breakpoint while the 3 remaining antibiotics have no established breakpoints due to their infrequent usage in treating *Pseudomonas* infections. All antibiotics of the ACSSuT group, of which SGI1 conveys resistance to, have MIC levels high enough to be considered resistant at clinically relevant levels.

Conclusions

- The *Pseudomonas* isolate has been identified by 16SrRNA sequencing to be a 99% match to *Pseudomonas fulva.*
- This isolate displays Multiple Drug Resistance (MDR) to at least 13 antibiotics across 10 classes.
- Comparison of MIC levels shows D9 to be at least as resistant to ACSSuT as known SGI1 containing organisms with the exception of spectinomycin.
- Shotgun and 454 sequencings reveal a large area of homology to the *Salmonella* Genomic Island 1 (SGI1) in a region that is known to convey MDR.
- Several genes located in this region have yet to be confirmed in D9.
- MDR is not likely conferred by a plasmid but may be caused by MDR genes on a SGI1 like region.

Acknowledgements

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