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Genetic context and mobilization of class 1 integrons in *Pseudomonas aeruginosa*: Are plasmids redundant?

PhD by Research

Supervisor Professor Hatch Stokes

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Abbreviations

°C	Degrees Celsius
3'-CS	Class 1 integron 3'- conserved segment
5'-CS	Class 1 integron 5'- conserved segment
aacA/C	Aminoglycoside acetyltransferase gene
aadA/B	Aminoglycoside adenylyltransferase gene
aph	Aminoglycoside phosphotransferase gene
A^R	Antibiotic resistance
attC	Gene cassette associated recombination site
attI	Integron associated recombination site
Austral	Hospital Universitario Austral
bla	β-lactamase gene
BLAST	Basic Local Alignment Search Tool
Bocagrande	Hospital de Bocagrande
bp	Base pair
CAS	Clínica Fundacion Amigos de la Salud
CCM	Clínica Central de Monteria
CH/ Concord	Concord Repatriation General Hospital
Clinicas	Hospital de Clínicas Jose de San Martín
Cm	Chloramphenicol
cml	Chloramphenicol exporter gene
dfrA	Dihydrofolate reductase gene
dH_2O	Distilled, sterilized water
DNA	Deoxyribonucleic acid
DR	Direct repeats
Durand	Hospital Carlos G Durand
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium Bromide

EtOH	Ethanol
Favaloro	Fundación Favaloro-Hospital Universitario
Garrahan	Hospital de Pediatria Prof Dr Juan P Garrahan
HCFA	Hospital Central de las Fuerzas Armadas
hr/s	Hour/s
ICE	Integrating Conjugative Elements
Inc	Incompatibility
intI1	Class 1 integron integrase gene
IR	Inverted Repeat
IS	Insertion Sequence
JPC	Clínica Jorge Piñeros Corpas
kb	Kilo base/s
Kennedy	Hospital Kennedy
Km	Kanamycin
LB agar	Luria-Bertani agar
LGT	Lateral Gene Transfer
M	Molar
Militar	Hospital Militar Central
min/m	Minutes
Mitre	Sanatorio de la Trinidad Mitre
mL	Micro litre/s
MLST	Multi Locus Sequence Typing
mM	Millimolar
MqH_2O	Mili Q water
MSU	Mid-Stream Urine
N/A	Not applicable
NCBI	National Centre for Biotechnology Information
ng	Nanogram/s
Occidente	Clínica de Occidente
OD	Optical density

Olaya	Políclinico Olaya
ORF/orf	Open Reading Frame
P_c	Cassette Promoter- Integron associated
PCR	Polymerase chain reaction
PFGE	Pulse Field Gel Electrophoresis
qac	Quaternary ammonium compound resistance gene
Quemados	Hospital de Quemados
res	Site-specific Resolution site
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions per minute
Samaritana	Hospital Universitario la Samaritana
SAN/ San Adventist	Sydney Adventist Hospital.
Santa Clara	Hospital Santa Clara
San Ignacio	Hospital Universitario San Ignacio
San Jeronimo	Hospital San Jerónimo
San Rafael	Hospital Universitario Clínica San Rafael
sec/s	Second/s
SPC	Clinica San Pedro Claver
sulI	Sulfonamide resistance gene
TAE	Tris-acetate
Tn	Transposon
tni	Transposition genes
tnpA	Transposase gene
tnpR	Resolvase gene
tra genes	Conjugal Transfer genes
Tunal	Hospital el Tunal
UV	Ultraviolet
V	Volt/s
Vol	Volume/s
w/v	weight/volume

Zayma	Clínica Zayma
Δ	Deletion of
µg	Microgram/s
µl	Micro litre/s
µM	Micromolar/s

Abstract

Antibiotic resistance is a global problem with some predicting a return to the pre antibiotic era where a bacterial infection was commonly fatal. *Pseudomonas aeruginosa* is one example of this problem. This bacterium is a major cause of infection especially in cystic fibrosis sufferers and in burns victims. The rising rates of adverse outcomes are partly a consequence of strains commonly displaying multi-drug resistance (MDR) profiles. MDR is driven by a number of intrinsic mechanisms in *P. aeruginosa* clinical isolates as well as by the capture of diverse resistance-mediating genes by Lateral Gene Transfer (LGT). LGT and intrinsic factors often act cooperatively to generate complex MDR phenotypes. While these complex interactions have been examined in a small number of isolates there has not been a comprehensive survey of strains on a global scale. Thus it is not clear what mechanisms and genes may be important in influencing the evolution of MDR at regional or global levels. Also, in some isolates, resistance profiles cannot always be explained by identifying the common resistance determining pathways, suggesting that additional mechanisms of resistance may be emerging in *P. aeruginosa*. The focus of this project was to comprehensively study the major mechanisms responsible for antibiotic resistance in *P. aeruginosa* strains from diverse geographical areas.

Pathogenic *P. aeruginosa* isolates from four countries (Australia and three South American countries) were characterized by PCR to identify mobile elements and their genetic context. Also, quantitative expression analysis for activity of several pathways that influence antibiotic resistance was assessed and culture experiments were conducted to test how

random movement of mobile elements during growth may influence resistance to some antibiotics.

Data presented in this thesis indicated that, in most strains, antibiotic resistance was being driven by changes in multiple pathways (including overexpression of AmpC and two efflux pumps) and by the presence or absence of genes acquired by Lateral Gene Transfer (LGT). Class 1 integrons, elements important in the spread of antibiotic resistance genes in Gram-negative bacteria, were most frequently recovered in South American countries. Many class 1 integrons were mapped to a specific location within the genome. Regardless of country of origin all these mapped integrons were found to be in the chromosome, often in Genomic islands, and not on a plasmid despite data in the literature implying the opposite. The association of class 1 integrons with genomic islands may be an important mechanism driving LGT in *P. aeruginosa*. Also, a newly emerging mechanism involving the insertion sequence IS*26* was identified that is capable of mobilizing resistance and other genes. This IS*26*-mediated mechanism may allow phenotype switching in clonal lines in a way that is likely to further exacerbate the treatment of infections mediated by *P. aeruginosa*.

Data presented here suggested that *P. aeruginosa* strains are evolving to become multidrug resistant in increasingly complex ways. This is occurring by single strains acquiring changes in numerous known pathways as well as by newly emerging resistance mechanisms in this species.

Publications arising from this work

- **Elena Martínez**, Javier Escobar Perez, Carolina Marquez, Elizabet Vilacoba, Daniela Centron, Aura L. Leal, Carlos Saavedra, Sandra Y. Saavedra, Catalina Tovar, Natasha Vanegas and Hatch Stokes. 'Emerging and existing mechanisms in generating diverse β-lactam resistance phenotypes in *Pseudomonas aeruginosa'*. *Journal of Global Antimicrobial Resistance* 2013. Volume 1, Issue 3: 135-142.

- **Elena Martínez,** Steven Djordjevic, H.W. Stokes and Piklu Roy Chowdhury. 'Mobilized Integrons: Team Players In the Spread of Antibiotic Resistance Genes'. Lateral Gene Transfer in Evolution, edited by Uri Gophna. Springer 2013.

- **Elena Martínez**, Carolina Marquez, Ana Ingold, John Merlino, Steven Djordjevic, Hatch Stokes and Piklu Roy Chowdhury. 'Diverse mobilized class 1 integrons are common in the chromosomes of pathogenic *Pseudomonas aeruginosa* clinical isolates'. *Antimicrob. Agents Chemother.* 2012 April 56: 2169-72

- Hatch Stokes, **Elena Martínez**, Piklu Roy Chowdhury and Steve Djordjevic. 'Class 1 integron-associated spread of resistance regions in *Pseudomonas aeruginosa*: plasmid or chromosomal platforms?' *Journal Antimicrobial Chemotherapy* 2012 March 67: 1799-800