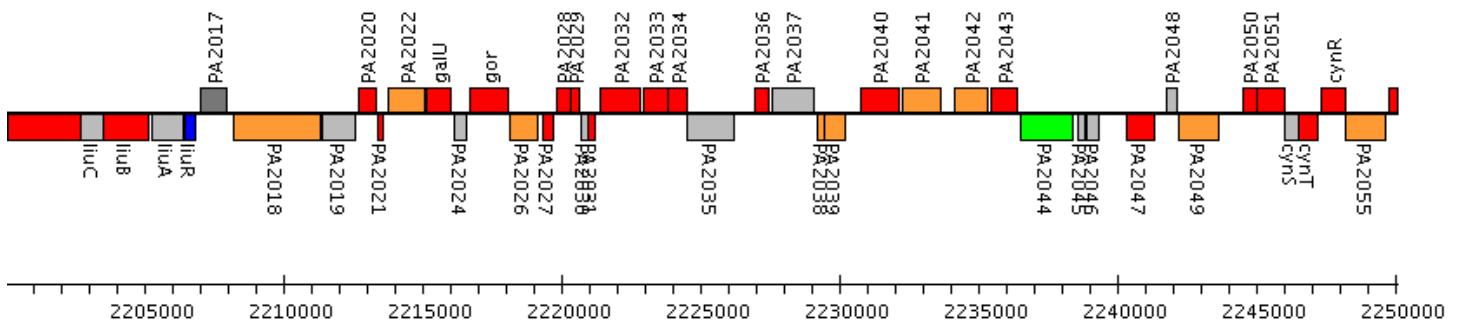
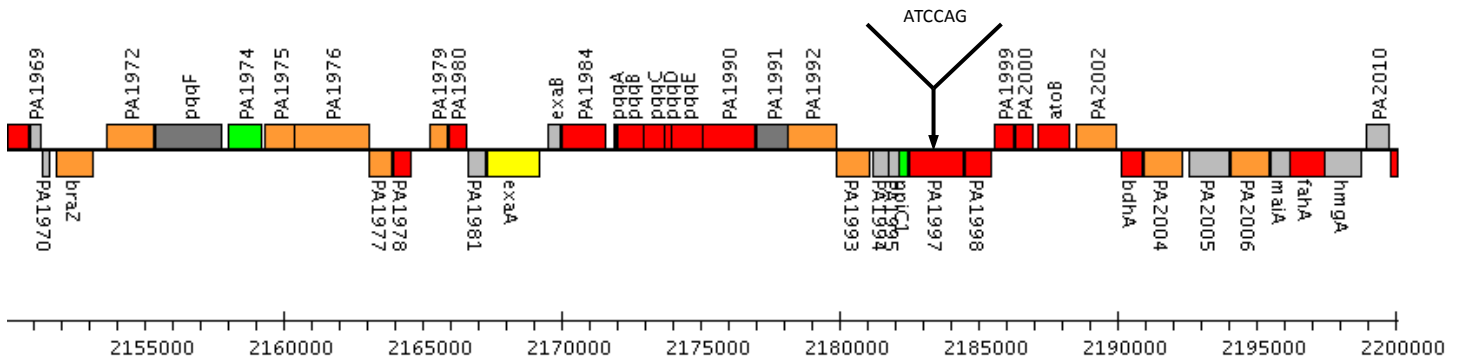
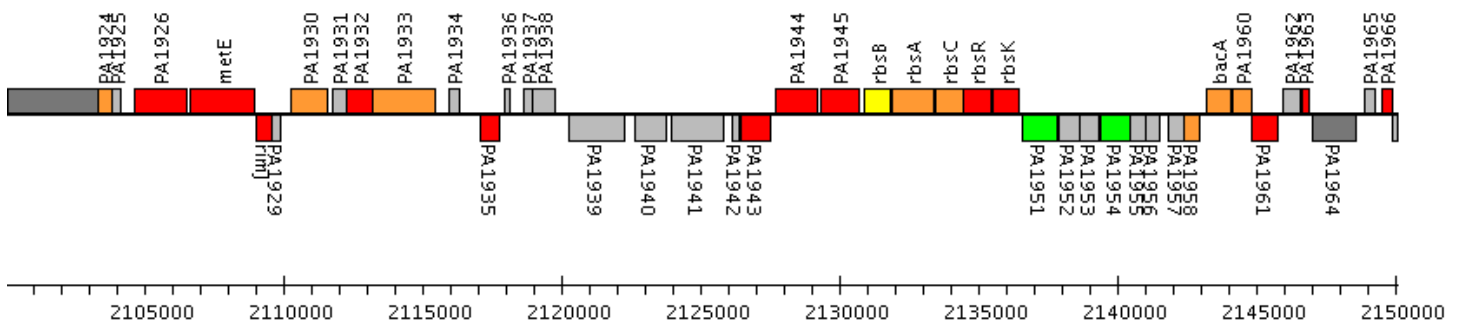
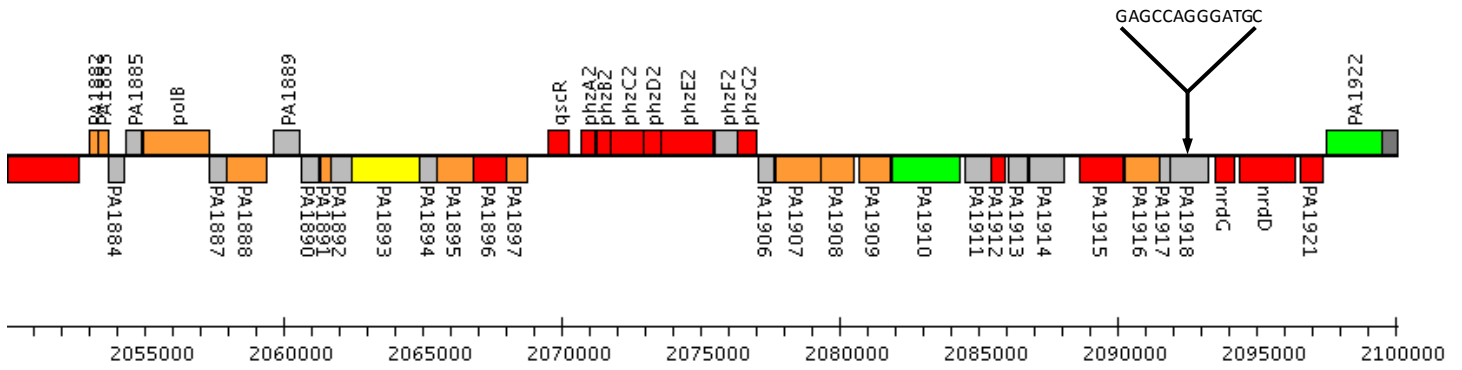


Figure S1. SDS-PAGE analysis of *P. aeruginosa* outer membrane proteins (OMPs) of PAO1 and PAOMS lineages evolved in the presence of meropenem. From left to right: Molecular Weight Marker, PAO1, PAO1 OprD mutant (PAOD1) and the 3 of PAO1 and PAOMS evolved lineages.



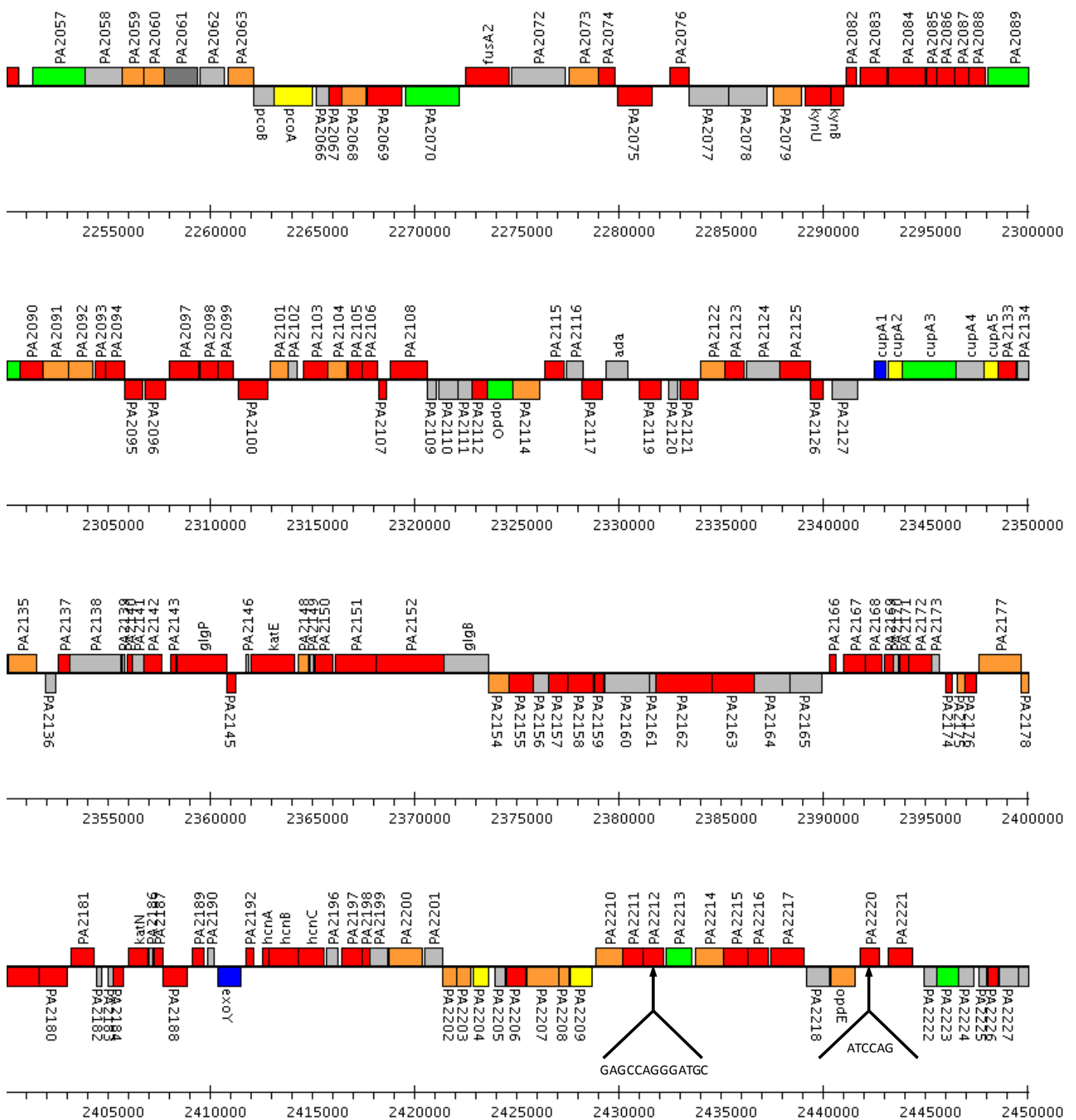


Figure S2. Representation of the deleted regions in PAO1.1-MER and PAO1.3-MER. Inverted repeats flanking the deleted regions in PAO1.1-MER (ATCCAG) and PAO1.3-MER (GAGCCAGGGATGC) are indicated. Template PAO1 genome was obtained from <http://www.pseudomonas.com>.

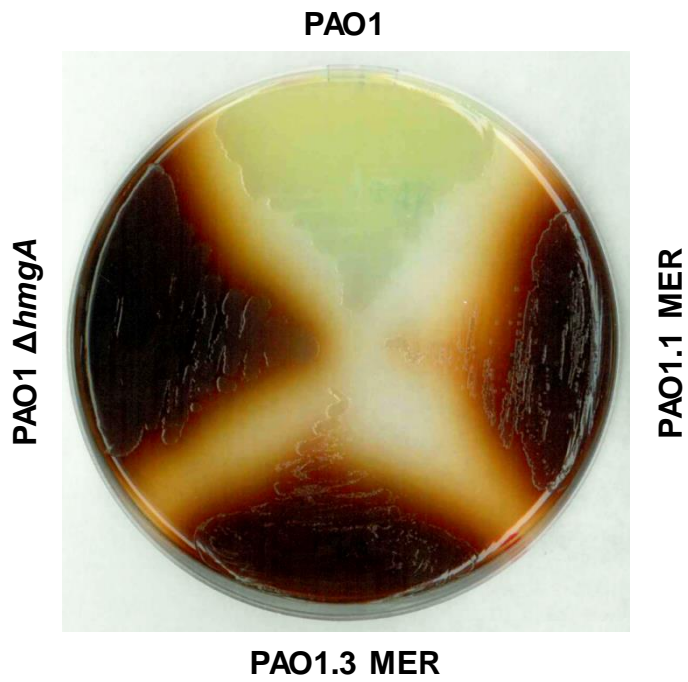


Figure S3. Pyomelanin hyperproduction. MH agar plate showing the pyomelanin hyperproduction phenotype of PAO1.1-MER and PAO1.3-MER. PAO1 is used as negative control and a $\Delta hmgA$ PAO1 derivative as positive control.

Table S1. Oligonucleotides used in this work.

Primer	Sequence (5' → 3')	PCR product size (bp)	Use	Source
oprD-F	CGCCGACAAGAAGAACTAGC	1413	<i>oprD</i> amplification and sequencing	[1]
oprD-R	GTCGATTACAGGATCGACAG			
PA1918-F	ACAAGACTCATACGATCGTAC	1000	Characterization of MER mutants deletion	This work
PA1918-Rint	GCGGTAGTAGTCCACCATC			
PA1997-F	GGTTATGCTTCCTGCATGTC	1381	Characterization of MER mutants deletion	This work
PA1997-Rint	CCTTGCAATTGCAGTTCGCC			
PA2212-F	CGTACGCCGACCAGGAAC	772	Characterization of MER mutants deletion	This work
PA2212-Rint	GTTGTCGCCGTTGTGCGG			
PA2220-F	GATCAGCTCCGCTGGTGAC	836	Characterization of MER mutants deletion	This work
PA2220-Rint	ACACGGACGTTACGTTGTC			
hmgA-F	GGGCTTGAGGATATCGG	1585	<i>hmgA</i> amplification and sequencing	[2]
hmgA-R	AGGCGACCCAGCTACGAGTG			
ampD-F	GTACGCCTGCTGGACGATG	910	Amplification and sequencing of AmpC regulator <i>ampD</i>	[3]
ampD-R	GAGGGCAGATCCTCGACCAG			
ampR-F	GTCGACCCAGTGCCTTCAGG	1400	Amplification and sequencing of AmpC regulator <i>ampR</i>	[3]
ampR-R	CTCGAGAGCGAGATCGTTGC			
dacB-F	CGACCATTCCGGCGATATGAC	1721	Amplification and sequencing of AmpC regulator <i>dacB</i>	[4]
dacB-R	CGCGTAATCCGAAGATCCATC			
nalB-F	CAGCGTGAAGGCGCTGCAC	790	Amplification and sequencing of <i>mexAB-oprM</i> regulator <i>mexR</i>	[5]
nalB-R	GAGCTGCTGCTCTCCGTCG			
nalC-F	TCAACCCTAACGAGAAACGCT	1150	Amplification and sequencing of <i>mexAB-oprM</i> regulator <i>nalC</i>	[6]
nalC-R	TCCACCTCACCGAACTGC			
nalD-F	GCGGCTAAAATCGGTACACT	1100	Amplification and sequencing of <i>mexAB-oprM</i> regulator <i>nalD</i>	[7]
nalD-R	ACGTCCAGGTGGATCTTGG			
nfxB-F	GCCTCCTGTCGCTCTTCCG	957	Amplification and sequencing of <i>mexCD-oprJ</i> regulator <i>nfxB</i>	[8]
nfxB-R	CTGTGCGAGGCACTTTGTGCG			
mexT-F	CTGTATCCGCCCATGCCTG	1126	Amplification and sequencing of <i>mexEF-oprN</i> regulator <i>mexT</i>	This work
mexT-R	GACGCCTCGTGCGGGTAG			
mexS-F	TGACAGGCATAGCCATTATC	1209	Amplification and sequencing of <i>mexEF-oprN</i> regulator <i>mexS</i>	This work
mexS-R	GGTCAACGATCTGTGGATC			
mvaT-F	CCACTCAGCACAGACAAGGT	440	Amplification and sequencing of <i>mexEF-oprN</i> regulator <i>mvaT</i>	This work
mvaT-R	GCAGAGGAGCCGATACAATC			
mexZ-F	ATTGGATGTGCATGGGTG	1000	Amplification and sequencing of <i>mexXY-oprM</i> regulator <i>mexZ</i>	[9]
mexZ-R	TGGAGATCGAAGGCAGC			
PA5471-F	GATCTACCGTTTCAATCACATGGAT	1600	Amplification and sequencing of <i>mexXY-oprM</i> regulator <i>PA5471</i>	[10]
PA5471-R	GGCCACCTCCTCGATTACCT			
gyrA1	TTATGCCATGAGCGAGCTGGGCAACGACT	364	<i>gyrA</i> amplification and sequencing	[11]
gyrA2	AACCGTTGACCAGCAGGTTGGGAATCTT			
gyrB3	AGCTCGCAGACCAAGGACAAG	600	<i>gyrB</i> amplification and sequencing	[11]
gyrB4	GGGCTGGGCGATGTAGATGTA			
parC1	ATGAGCGAACTGGGGCTGGAT	208	<i>parC</i> amplification and sequencing	[11]
parC2	ATGGCGGCGAAGGACTTGGGA			
parE1	CGGCGTTCGTCTCGGGCGTGGTGAAGGA	592	<i>parE</i> amplification and sequencing	[11]
parE2	TCGAGGGCGTAGTAGATGTCCTTGCCGA			
rpsL _{RNA} -F	GCTGCAAACTGCCCGCAACG	250	Control house Keeping gene RT-PCR	[11]
rpsL _{RNA} -R	ACCCGAGGTGTCCAGCGAACC			
ampC _{RNA} -F	GGGCTGGCCTCGAAAGAGGAC	246	<i>ampC</i> expression RT-PCR	[12]
ampC _{RNA} -R	GCACCGAGTCGGGGAAGTCA			
mexB _{RNA} -F	CAAGGGCGTCCGTGACTTCCAG	273	<i>mexB</i> expression RT-PCR	[11]
mexB _{RNA} -R	ACCTGGGAACCGTCGGGATTGA			

mexD _{RNA} -F	GGAGTTCGGCCAGGTAGTGCTG	236	mexD expression RT-PCR	[11]
mexD _{RNA} -R	ACTGCATGTCCCTCGGGGAAGAA			
mexF _{RNA} -F	CGCCTGGTCACCGAGGAAGAGT	254	mexF expression RT-PCR	[11]
mexF _{RNA} -R	TAGTCCATGGCTTGGCGGAAGC			
mexY _{RNA} -F	TGGAAGTGCAGAACCGCCTG	270	mexY expression RT-PCR	[11]
mexY _{RNA} -R	AGGTCAGCTTGGCCGGGTC			

[1] Gutiérrez O, Juan C, Cercenado E, Navarro F, Bouza E, Coll P, et al. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Spanish hospitals. *Antimicrob Agents Chemother.* 2007; 51(12):4329–35.

[2] Rodríguez-Rojas A, Mena A, Martín S, Borrell N, Oliver A, Blázquez J. Inactivation of the *hmgA* gene of *Pseudomonas aeruginosa* leads to pyomelanin hyperproduction, stress resistance and increased persistence in chronic lung infection. *Microbiology.* 2009; 155(4):1050-7.

[3] Juan C, Maciá MD, Gutiérrez O, Vidal C, Pérez JL, Oliver A. Molecular mechanisms of beta-lactam resistance mediated by AmpC hyperproduction in *Pseudomonas aeruginosa* clinical strains. *Antimicrob Agents Chemother.* 2005; 49(11):4733-8.

[4] Moya B, Dötsch A, Juan C, Blázquez J, Zamorano L, Haussler S, et al. β -Lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. *PLoS Pathog.* 2009; 5(3):e1000353.

[5] Cabot G, Ocampo-Sosa AA, Domínguez MA, Gago JF, Juan C, Tubau F, et al. Genetic Markers of Widespread Extensively Drug-Resistant *Pseudomonas aeruginosa* High-Risk Clones. *Antimicrob Agents Chemoter.* 2012; 56(12):6349-57.

[6] Llanes C, Hocquet D, Vogne C, Benali-Baitich D, Neuwirth C, Plésiat P. Clinical strains of *Pseudomonas aeruginosa* overproducing MexAB-OprM and MexXY efflux pumps simultaneously. *Antimicrob Agents Chemother.* 2004; 48(5):1797–802.

[7] Sobel ML, Hocquet D, Cao L, Plésiat P, Poole K. Mutations in PA3574 (*nalD*) lead to increased MexAB-OprM expression and multidrug resistance in laboratory and clinical isolates of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2005; 49(5):1782–6

[8] Mulet X, Macià MD, Mena A, Juan C, Pérez JL, Oliver A. Azithromycin in *Pseudomonas aeruginosa* Biofilms: Bactericidal Activity and Selection of *nfxB* Mutants. *Antimicrob Agents Chemoter.* 2009. 53(4):1552-60.

[9] Sobel ML, McKay GA, Poole K. Contribution of the MexXY multidrug transporter to aminoglycoside resistance in *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemoter.* 2003; 47(10):3202–7.

[10] Morita Y, Sobel ML, Poole K. Antibiotic Inducibility of the MexXY Multidrug Efflux System of *Pseudomonas aeruginosa*: Involvement of the Antibiotic-Inducible PA5471 Gene Product. *Journal of Bacteriology.* 2006; 188(5):1847-55.

[11] Oh H, Stenhoff S, Jalal S, Wretling B. Role of efflux pumps and mutations in genes for topoisomerases II and IV in fluoroquinolone-resistant *Pseudomonas aeruginosa* strains. *Microb Drug Resist.* 2003; 9(4):323–328.

[12] Juan C, Moya B, Perez JL, Oliver A. Stepwise upregulation of the *Pseudomonas aeruginosa* chromosomal cephalosporinase conferring high level beta-lactam resistance involves three AmpD homologues. *Antimicrob Agents Chemother.* 2006; 50(5):1780–7.