

Supplementary Methods, Results, Figures, Tables and References for Hernandez *et al.*

Detecting emerging bacterial pathogens

Sample collection and processing

Eight sampling sites on the Fal estuary and English Channel coast near Falmouth (Cornwall, United Kingdom) were selected for water and sediment sampling. Four sites were estuarine (Mylor, Flushing, Penryn and Falmouth Harbour) and four truly coastal (Castle Beach, Gyllyngvase Beach, Swanpool Beach, and Maenporth Beach) (Figure S1). Samples from each site were collected on 21 June 2017 and 6 July 2017. Each sampling effort consisted of collecting water (2*50 mL) and upper 1cm layer of sediment (~25 g) from all eight sites within a time span of two hours around low-tide. Samples were collected using sterile 50mL centrifuge tubes. All samples were kept on ice during transport and processed in laboratory within an hour from collection.

Each water sample was centrifuged at 500 rpm at 4°C for 15 minutes (2 x 50 mL) to pellet sediment particles. The supernatants were then transferred to new sterile 50mL centrifuge tubes and spun down at 3500 rpm at 4°C for 30 minutes to pellet bacteria. The supernatant was discarded and the pellet in each tube resuspended in 500µL of sterile M9 buffer. Resuspensions from the two tubes per sample were then combined in a 1.5 mL Eppendorf tube to obtain a total concentrated water sample of 1mL. Bacteria were extracted from sediments by adding 10g of sediment with 10mL of sterile M9 buffer. Samples were vortexed at 3000 rpm for 2 minutes, and then centrifuged at 500 rpm at 4°C for 15 minutes to pellet sediment particles. The supernatant was then centrifuged as above and resuspended in 1mL of M9 buffer. All samples were kept at 4°C or on ice at all times.

Flow cytometry

Concentrated water and sediment wash samples were analysed for bacterial load with the use of flow cytometry. For each sample (200 µL sample + 20 µL 2.5x SybrGold), 10 µL was analyzed for number of events using low flow rate (14 µL /min) in a BD Accuri C6 Plus flow cytometer (BD

Biosciences, San Jose, CA). The BD Accuri C6 Plus is equipped with a blue and red laser, two light scatter detectors, and four fluorescence detectors. Bacteria cells were distinguished from non-biological particles using 533/30 filter in FL1 (indicative of nucleic acid content), 670 LP in FL3 (indicative of chlorophyll content), and FSC (indicative of cell size). Data were analyzed using the BD Accuri C6 software. A non-dye sample was included to control for auto-fluorescence.

Culturable Bacteria

100µL of each water and sediment wash sample was plated onto both LB agar (Fisher BioReagents) and Coliform agar (Millipore Sigma) and incubated overnight at 37°C. CFU per plate were counted after 24 hr incubation. Colonies on coliform agar were scored as dark-blue (*Escherichia coli*), pink ('coliform'), or white/transparent ('other'). Units are reported in CFU/100mL for water samples and CFU/10g for sediment samples.

Galleria mellonella assay for environmental samples

Final instar waxmoth (*Galleria mellonella*) larvae (~220mg each) were purchased from Livefood UK (<http://www.livefood.co.uk>), stored in the dark at 4°C, and used within two weeks (all experiments utilized larvae from the same batch). A 100µL Hamilton syringe (Sigma-Aldrich Ltd) with 0.3 x 13mm needle (BD Microlance 3) was used to inject larvae with 10µL of sample into the last left proleg. New sterile needles were used for injection of each sample, and the syringe was cleaned between sample injections with 70% ethanol and sterile M9 buffer. All water and sediment wash samples (8 locations x 2 timepoints) were assayed in one experiment using 20 *Galleria* per sample. Three negative controls were used for each experiment: a no-injection control (to control for background larvae mortality), a buffer control in which larvae were injected with 10µL of M9 buffer (to control for impact of physical trauma), and a filter-sterilized sample control in which larvae were injected with filter-sterilized samples (0.22 µm syringe filters, Thermo Fisher) to control for any toxic chemicals present in the sample. Before injection, the *Galleria* larvae were separated into Petri dishes in groups of 20 and anesthetized by placing

on ice for approximately 30 minutes before injection. After injection, Petri dishes were incubated at 37°C and inspected at 24, 48 and 72 hours post-injection to record morbidity and mortality. *Galleria* larvae were considered infected if they expressed dark pigmentation (melanisation) after inoculation. Larvae were scored as dead if they did not respond to touch stimuli by blunt sterile forceps.

Galleria mellonella assay for individual clones

To isolate and identify responsible pathogens, selected samples showing high *Galleria* mortality were inoculated and incubated again as described above. Eight live larvae showing signs of infection (melanisation) were selected at random from each sample group and dissected for haemocoel collection. Before haemocoel collection, the *Galleria* larvae were anesthetized on ice for 30 minutes and the site of dissection was sterilized with 70% ethanol. Sterile micro-scissors were used to remove the last left proleg, and a drop of haemocoel was allowed to exude from the larvae before being collected with a pipette. Approximately 5-15µL of haemocoel was collected per larva and diluted into 500µL of sterile M9 buffer in an Eppendorf tube. Serial dilutions of each sample were plated onto LB- and coliform agar plates and incubated overnight at 37°C. A single clone per sample was picked from these plates and grown for 24 hours in 5mL of LB broth at 37°C at 180 rpm. Overnight cultures were diluted in broth to a turbidity equivalent to a McFarland standard of 0.5 at 625 nm as measured by spectrophotometry (Bibby Scientific Limited, Staffordshire, UK). A serial dilution was plated on LB agar and incubated for 24 hours at 37°C. LB agar plate colony counts were used to calculate CFU/mL. Individual clones were used to inoculate groups of 20 *Galleria* larvae with 10µL of 1x10² CFU, 1x10⁴ CFU, and 1x10⁶ CFU. Larvae were incubated as described in the previous section and morbidity and mortality was recorded hourly after an initial 10-hour period for a period of 37 hours allowing for construction of survival plots and calculation of the 50% lethal dose value (LD₅₀).

Whole Genome Sequencing

DNA isolation, Illumina HiSeq sequencing and basic bioinformatics was performed through the MicrobeNG program in Birmingham, UK. Vials containing beads inoculated with liquid culture were washed with extraction buffer containing lysostaphin and RNase A, incubated for 25 min at 37°C. Proteinase K and RNaseA were added and incubated for 5 min at 65°C. Genomic DNA was purified using an equal volume of SPRI beads and resuspended in EB buffer. DNA was quantified in triplicates with the Quantit dsDNA HS assay in an Eppendorff AF2200 plate reader. Genomic DNA libraries were prepared using Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: two nanograms of DNA instead of one were used as input, and PCR elongation time was increased to 1 min from 30 seconds. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system. Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche light cycler 96 qPCR machine. Libraries were sequenced on the Illumina HiSeq using a 250bp paired end protocol.

Bioinformatics

Reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 (Bolger et al 2014). De novo assembly was performed on samples using SPAdes version 3.7 (Bankevich et al 2012), and contigs were annotated using Prokka 1.11 (Seemann 2014). Kraken was used to indicate the likely species classification of our isolates (Wood and Salzberg 2014). For species with established MLST schemes, the sequence type of each isolate was identified using the Centre for Genomic Epidemiology MLST tool (Larsen et al 2012). Other isolates belonging to the same sequence types were found and accessed using databases Enterobase (Alikhan et al 2018) (*E. coli*) and (Jolley and Maiden 2010) (*P. aeruginosa*). Whole genome alignments were constructed using Progressive Mauve (Darling et al 2010). Percentage similarity was calculated using the number of SNPs found between the query isolate and the reference sequence. In the case of *V. injenesis* where a closed reference genome was not available, nucleotide identity was

confirmed using a core genome alignment to closely related draft genome. Core genomes were constructed using Roary 3.12.0 (Page et al 2015).

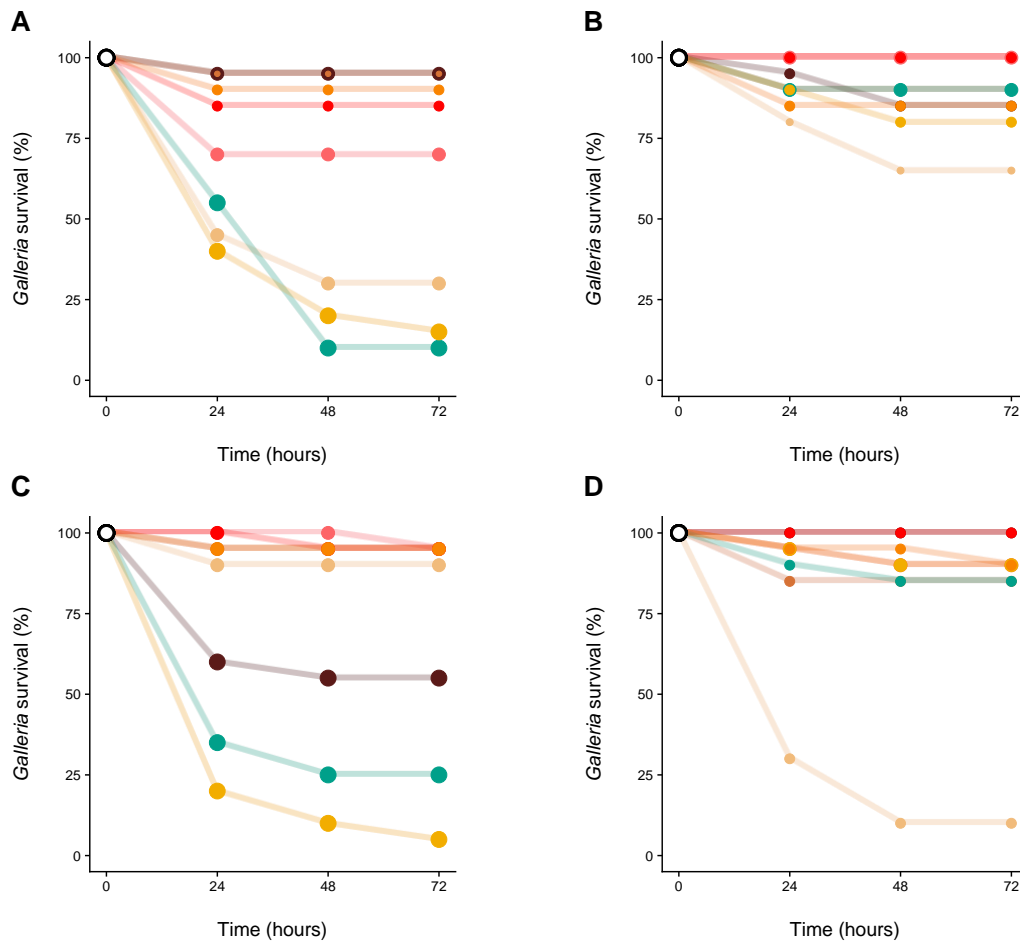
Differences in gene content (95% nucleotide identity threshold) between the *V. injenensis* reference strain and the *V. injenensis* clone isolated in this study were also identified using Roary 3.12.0. The presence of PCAMU-SGI1 in the *P. mirabilis* isolate was assessed using BLAST (Altschul et al 1990). Assemblies were screened for antimicrobial resistance related genes and virulence related genes using ABRicate (<https://github.com/tseemann/abricat>) using 90% and 75% nucleotide identity, and 80% length identity cut offs. The CARD database was used to identify AMR genes (McArthur et al 2013), and the VFDB virulence factor database was used to find putative virulence factors (Chen et al 2015). BRIG version 0.95 was used to represent circular draft assemblies and indicate the position of AMR and virulence genes on each contig (Alikhan et al 2011). Contigs were ordered by whole genome alignment against a reference genome using progressive mauve (Darling et al 2010). The *E. coli* isolate was aligned against K-12 substr. MG1655 (NC_000913.3), the *P. mirabilis* isolate was aligned against HI4320 (NC_010554.1), and the *P. aeruginosa* against PA01 (NC_002516.2). The *V. injenensis* genome could not be aligned to a closed reference genome and so the contigs could not be ordered or separated by chromosome.



Figure S1: Sampling sites around Falmouth, Cornwall, U.K. (50°.16' N 5°.10' W).

Supplementary Results

Figure S2. *Galleria* survival (%; 20 individuals per group) 24, 48 and 72 hours post-injection with 10 μ l of 100-fold concentrated water (panel B, 21st June, panel D, 6st July) or sediment wash (see Supplemental Methods) (panel A, 21st June, panel C, 6st July). Different colours denote different localities.



Correlation of virulence with measures of bacterial density

Bacterial density in water and sediment wash samples was quantified in four ways: 1) total density by flow cytometry, 2) total colony counts on LB agar, 3) total colony counts on coliform agar (dark-blue, pink and white/transparent colonies that include gram-negative, non-enteric bacteria) and 4) *E. coli* and coliform colony counts (only dark-blue and pink colonies on coliform agar) (Figure S3). Generalized linear mixed effects models (glmer function from the 'glmm' R package) were used to test the additive effects of bacterial cell density ($\log_{10} + 1$) and sample type (sediment versus water) on *Galleria* mortality with a binomial error structure and random intercepts fitted for sampling date, and location nested within sampling date. Separate models were fitted for each of the four different measures of bacterial cell density. Crucially, we found that *Galleria* mortality was strongly, and positively, dependent on cell density for all different density measures (Figure S4 and Table S1). We also found that *Galleria* mortality was significantly lower (for a given bacterial count) when larvae were injected with water samples compared to sediment samples. This could point at the sediment being inhabited by different, more pathogenic bacteria. The exception to this was formed by estimates based on flow cytometry (Table S1), potentially because culture-based approaches reflect total bacterial density more reliably. There was a strong correlation between coliform cells and total counts on coliform agar with two notable exceptions: Castle Beach water and Penryn sediment (both sampled 6 July); both samples had high mortality, presumably due to these non-coliform bacteria.

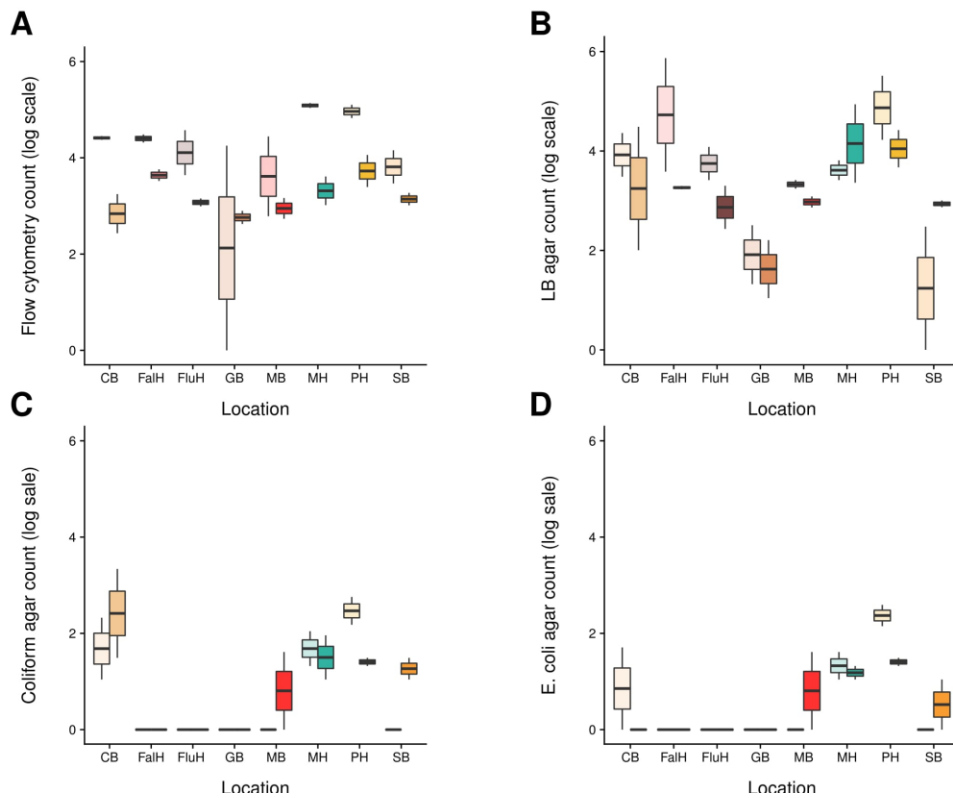


Figure S3. Boxplot summarizing variation in bacterial densities (expressed as \log_{10} cells per 100 ml) across eight different locations (see text) and sample types (water versus sediment, where water samples are depicted first per location). Bacterial cell densities were enumerated using: A) flow cytometry, (B) total LB agar counts, (C) total coliform agar counts and (D) *E. coli* and coliform counts (blue and pink colonies on coliform agar).

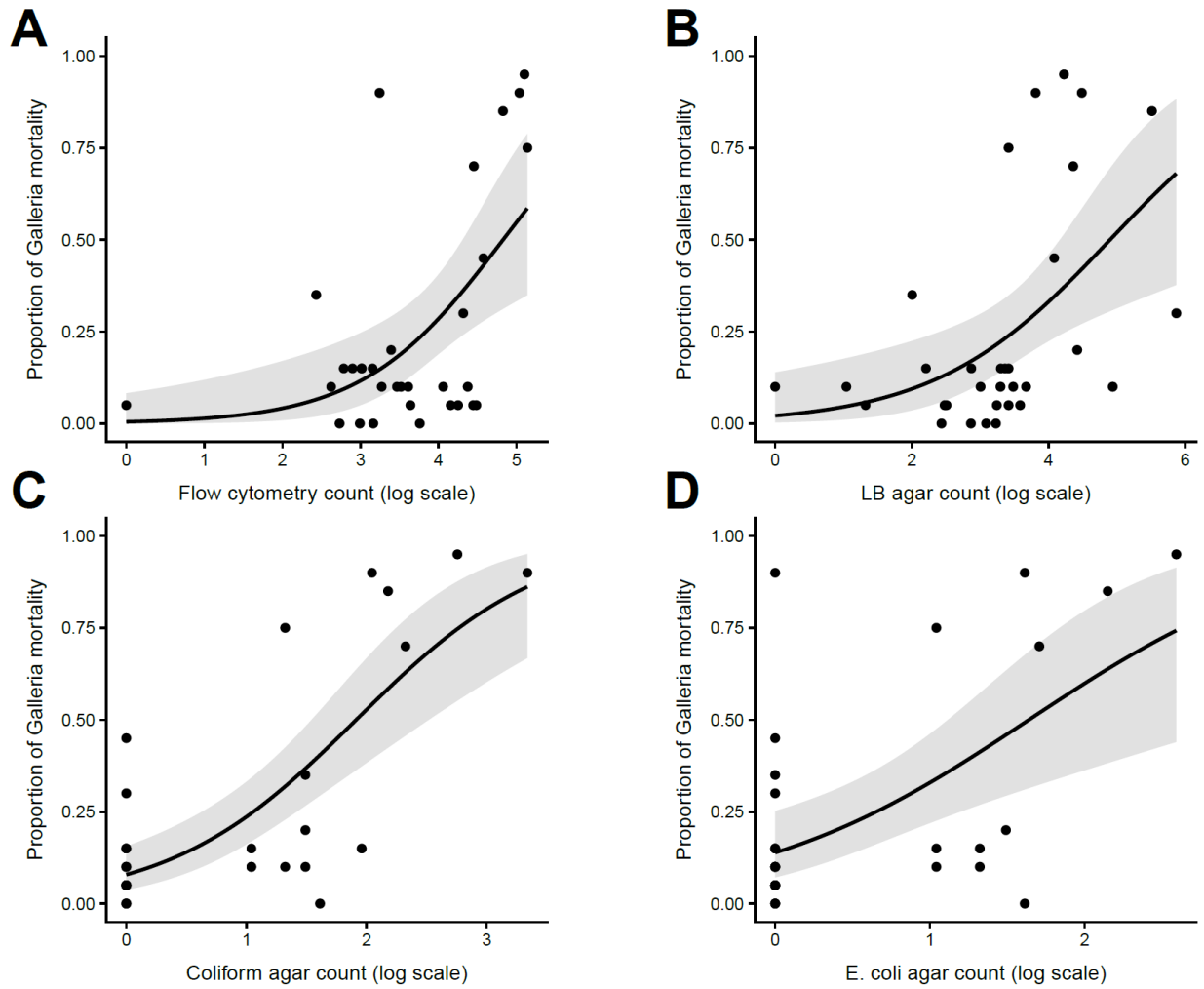


Figure S4. Plots depicting the relationship between *Galleria* mortality and bacterial cell density, estimated using: (A) flow cytometry, (B) total LB cell counts, (C) total coliform agar counts and (D) *E. coli* and coliform counts (blue and pink colonies on coliform agar). Lines and shaded area depict the fitted relationships \pm standard error (see Table S1 for parameter estimates).

Table S1. Results of the generalized linear mixed effects models (glmer) testing the additive effects of bacterial cell density ($\log_{10} + 1$) and sample type (sediment versus water) on *Galleria* mortality with a binomial error structure and random intercepts fitted for sampling date, and location nested within sampling data. Separate models were fitted for different estimates of bacterial density (1-4). The most parsimonious model was arrived at by sequentially deleting terms and comparing model fits using χ^2 -tests of likelihood ratios.

1. Flow cytometry	Parameter estimate \pm SE [§]	χ^2 -test
Intercept	-4.02 \pm 1.01 ^{***} , z = -3.99	$\chi^2_{1,3} = 25.34, p < 0.001$ $\chi^2_{1,4} = 0.001, p = 0.97$
Density	0.63 \pm 0.23 ^{**} , z = 2.78	
Sample	-0.01 \pm 0.34 ^{NS} , z = -0.04	
Date	Standard deviation <0.001	
Date/Location	Standard deviation = 0.61	
2. LB agar counts		
Intercept	-3.00 \pm 0.49 ^{***} , z = -6.11	$\chi^2_{1,4} = 14.48, p < 0.001$ $\chi^2_{1,4} = 9.34, p < 0.01$
Density	0.47 \pm 0.12 ^{***} , z = 3.85	
Sample	-0.62 \pm 0.20 ^{**} , z = -3.04	
Date	Standard deviation <0.001	
Date/Location	Standard deviation = 0.62	
3. Total coliform counts		
Intercept	-1.97 \pm 0.17 ^{***} , z = -11.73	$\chi^2_{1,4} = 48.53, p < 0.001$ $\chi^2_{1,4} = 43.39, p < 0.001$
Density	0.78 \pm 0.09 ^{***} , z = 8.94	
Sample	-0.92 \pm 0.20 ^{***} , z = -4.62	
Date	Standard deviation <0.001	
Date/Location	Standard deviation < 0.001	
4. <i>E. coli</i> counts		
Intercept	-1.76 \pm 0.23 ^{***} , z = -7.76	$\chi^2_{1,4} = 11.32, p < 0.001$ $\chi^2_{1,4} = 7.99, p < 0.01$
Density	0.62 \pm 0.09 ^{***} , z = 3.82	
Sample	-0.57 \pm 0.20 ^{**} , z = -2.82	
Date	Standard deviation <0.001	
Date/Location	Standard deviation = 0.52	

§ The 'Sample' effect in the fitted models provides a measure of the extent to which water and sediment differ in their effect on *Galleria* mortality, where negative values indicate a lower detrimental effect of water on *Galleria* mortality (i.e., smaller estimated intercept) compared to sediment (Intercept). $P < 0.001$ ^{***}, $P < 0.01$ ^{**}.

Isolation of pathogenic clones

Individual pathogenic clones were isolated from four samples showing high mortality, genomes of which are described in the main text. The Penryn Harbour sediment (sampled 6 July) yielded a white coloured clone on coliform agar and a 'bubble-wrap' colony morphology on LB agar which was identified as *Proteus mirabilis*. Interestingly, this clone did not cause the usual melanisation in *Galleria* but instead, the larvae turned brown, indicating a different pathogenicity mechanism. Castle Beach sediment (sampled 21 June) yielded a transparent clone on LB agar which was identified as *Vibrio injenensis*. Castle Beach water (sampled 6 July) yielded a bright green clone on LB agar identified as *Pseudomonas aeruginosa* ST-667. Mylor Harbour sediment (sampled 6 July) yielded a dark-blue clone on coliform agar, which was identified as *Escherichia coli* ST-3304.

Tables with virulence- and antibiotic resistance genes found in the four genome-sequenced isolates. Hits tabulated in white are >90% nucleotide similarity (>80% coverage) and hits tabulated in grey are >75% nucleotide similarity (>80% coverage).

Table S2 Virulence genes detected in *Proteus mirabilis* Penryn Harbour clone.

gene name	nt identity (%)	coverage (%)	acc. nr.	description
<i>flhC</i>	76.11	93.99	YP_001006783	flagellar biosynthesis transcription activator

Table S3 Antibiotic resistance genes detected in *Proteus mirabilis* Penryn Harbour clone.

gene name	nt identity (%)	coverage (%)	acc. nr.	description
<i>sul1</i>	100	100	JF969163:1054-1894	a sulfonamide resistant dihydropteroate synthase of Gram-negative bacteria linked to other resistance genes of class 1 integrons.
<i>tet(J)</i>	98.16	100	AF038993:1-1198	a tetracycline efflux protein expressed in Gram-negative bacteria (<i>Escherichia</i> , <i>Morganella</i> and <i>Proteus</i>).
<i>CRP</i>	80.41	99.84	AP009048:4153664-4154297	a global regulator that represses MdtEF multidrug efflux pump expression.
<i>dfrA15</i>	84.63	100	KF534911:40-514	an integron-encoded dihydrofolate reductase found in <i>Vibrio cholerae</i>
<i>plasmid-encoded_cat_(pp-cat)</i>	79.33	94.4	D16171:383-1043	a plasmid-encoded variant of the cat gene found in <i>Photobacterium damsela</i> subsp. <i>piscicida</i>

Table S4 Virulence genes detected in *Vibrio injenensis* Castle Beach clone.

<i>rtxC*</i>	96.97	100	NP_231093	RTX toxin activating protein
<i>hcp-2</i>	83.65	99.81	NP_232418	type VI secretion system substrate
<i>luxS</i>	75.94	82.47	NP_230208	S-ribosylhomocysteinase
<i>rtxB</i>	82.17	99.49	NP_231091	RTX toxin transporter
<i>rtxD</i>	79.69	99.93	NP_231090	RTX toxin transporter
<i>vipA/mglA</i>	75	99.8	NP_232508	type VI secretion system tubule-forming protein
<i>vipB/mglB</i>	79.53	96.75	NP_232509	type VI secretion system tubule-forming protein
<i>vscN2</i>	77.37	99.92	NP_800848	type III secretion system ATPase

*= The *rtxA* gene which is the exotoxin is present at 88% similarity but in two parts (coverage 74.62% and 24.52%) suggesting it might be inactive. The Castle Beach clone carries a zonula occludens toxin (Zot) known to increase mammalian gut permeability (74% amino acid similarity to the fish pathogen *V. anguillarum* (Castillo et al 2017) which is not present in the *V. injenensis* Type Strain.

Table S5 Antibiotic resistance genes detected in *Vibrio injenensis* Castle Beach clone.

gene name	nt identity (%)	coverage (%)	acc. nr.	description
<i>tet34</i>	75.05	100	AB061440:306-771	causes the activation of Mg ²⁺ -dependent purine nucleotide synthesis which protects the protein synthesis pathway.
<i>CRP</i>	79.84	99.53	AP009048:4153664-4154297	a global regulator that represses MdtEF multidrug efflux pump expression.

Table S6 Virulence genes detected in *Pseudomonas aeruginosa* Castle Beach clone.

gene name	nt identity (%)	coverage (%)	acc. nr.	description
<i>alg44</i>	99.66	100	NP_252232	alginate biosynthesis protein <i>Alg8</i>
<i>alg8</i>	99.8	100	NP_252231	alginate-c5-mannuronan-epimerase <i>AlgG</i>
<i>algA</i>	98.82	100	NP_252241	phosphomannose isomerase / guanosine 5'-diphospho-D-mannose pyrophosphorylase
<i>algB</i>	99.19	100	NP_254170	two-component response regulator
<i>algC</i>	99.14	100	NP_254009	phosphomannomutase
<i>algD</i>	99.24	100	NP_252230	GDP-mannose 6-dehydrogenase
<i>algE</i>	99.66	100	NP_252234	alginate biosynthetic protein <i>AlgK</i> precursor
<i>algF</i>	99.69	100	NP_252240	alginate o-acetyltransferase
<i>algG</i>	99.63	100	NP_252235	outer membrane protein <i>AlgE</i>
<i>algI</i>	99.49	100	NP_252238	alginate o-acetyltransferase
<i>algJ</i>	99.15	100	NP_252239	alginate o-acetyltransferase
<i>algK</i>	99.72	100	NP_252233	alginate biosynthesis protein <i>Alg44</i>
<i>algL</i>	99.28	100	NP_252237	poly(beta-d-mannuronate) lyase precursor
<i>algP/algR3</i>	97.17	98.87	NP_253940	alginate regulatory protein
<i>algQ</i>	99.17	100	NP_253942	alginate regulatory protein
<i>algR</i>	99.6	100	NP_253948	alginate biosynthesis regulatory protein <i>AlgR</i>
<i>algU</i>	99.14	100	NP_249453	alginate biosynthesis protein <i>AlgZ/FimS</i>
<i>algW</i>	98.72	100	NP_253136	<i>AlgW</i> protein [Alginate regulation]
<i>algX</i>	99.16	100	NP_252236	alginate biosynthesis protein
<i>algZ</i>	99.35	100	NP_253949	sigma factor <i>AlgU</i> [Alginate (VF0091)] [<i>Pseudomonas aeruginosa</i> PAO1]
<i>aprA</i>	99.24	100	NP_249940	alkaline metalloproteinase precursor

<i>chpA</i>	98.66	99.92	NP_249104	still frameshift probable component of chemotactic signal transduction system
<i>chpB</i>	98.64	100	NP_249105	probable methylesterase
<i>chpC</i>	98.82	100	NP_249106	probable chemotaxis protein
<i>chpD</i>	99.12	100	NP_249107	probable transcriptional regulator
<i>chpE</i>	98.86	100	NP_249108	probable chemotaxis protein
<i>clpV1</i>	98.93	100	NP_248780	type VI secretion system AAA+ family ATPase
<i>dotU1</i>	99.33	100	NP_248768	type VI secretion system protein
<i>exoT</i>	99.42	100	NP_248734	type III secretion system effector
<i>exoU</i>	99.42	100	AAC16023	type III secretion system effector
<i>exsA</i>	98.69	100	NP_250404	type III secretion system regulatory protein
<i>exsB</i>	98.07	100	NP_250403	type III secretion system pilotin
<i>exsC</i>	97.49	100	NP_250401	type III secretion system regulatory protein
<i>exsD</i>	99.16	100	NP_250405	type III secretion system regulatory protein
<i>exsE</i>	96.75	100	NP_250402	type III secretion system regulatory protein
<i>fha1</i>	96.19	96.99	NP_248771	type VI secretion system forkhead-associated protein
<i>fimT</i>	97.06	100	NP_253239	type 4 fimbrial biogenesis protein
<i>fimU</i>	99.61	100	NP_253240	type 4 fimbrial biogenesis protein
<i>fimV</i>	98.27	100	NP_251805	putative Type IV pili related protein
<i>fleI/flag</i>	99.73	100	NP_249784	flagellar protein
<i>fleN</i>	99.29	100	NP_250145	flagellar synthesis regulator FleN
<i>fleP</i>	99.33	100	NP_249787	flagellar protein FlIT [Deoxyhexose linking sugar 209 Da capping structure (A1138)]
<i>fleQ</i>	99.19	100	NP_249788	transcriptional regulator
<i>fleR</i>	98.94	100	NP_249790	two-component response regulator
<i>fleS</i>	99.09	100	NP_249789	two-component sensor
<i>flgA</i>	99.28	100	NP_252040	flagellar basal body P-ring biosynthesis protein
<i>flgB</i>	99.75	100	NP_249768	flagellar basal body rod protein
<i>flgC</i>	100	100	NP_249769	flagellar basal-body rod protein
<i>flgD</i>	99.16	100	NP_249770	flagellar basal-body rod modification protein
<i>flgE</i>	98.56	100	NP_249771	flagellar hook protein
<i>flgF</i>	99.07	100	NP_249772	flagellar basal-body rod protein
<i>flgG</i>	99.24	100	NP_249773	flagellar basal-body rod protein
<i>flgH</i>	98.85	100	NP_249774	flagellar L-ring protein precursor
<i>flgI</i>	99.46	100	NP_249775	flagellar P-ring protein precursor
<i>flgJ</i>	99.25	100	NP_249776	flagellar rod assembly protein/muramidase
<i>flgK</i>	99.12	100	NP_249777	flagellar hook-associated protein 1

<i>flgL</i>	99.32	100	NP_249778	flagellar hook-associated protein 3
<i>flgM</i>	98.46	100	NP_252041	negative regulator of flagellin synthesis
<i>flgN</i>	98.73	100	NP_252042	flagella synthesis protein
<i>flhA</i>	99.01	100	NP_250143	flagellar biosynthesis protein
<i>flhB</i>	98.94	100	NP_250140	flagellar biosynthetic protein
<i>flhF</i>	98.92	100	NP_250144	flagellar biosynthesis protein
<i>fliA</i>	99.46	100	NP_250146	flagellar biosynthesis sigma factor
<i>fliC</i>	99.52	100	NP_249783	B-type flagellin
<i>fliD</i>	98.95	100	NP_249785	flagellar capping protein
<i>fliE</i>	99.7	100	NP_249791	flagellar hook-basal body complex protein
<i>fliF</i>	99.56	100	NP_249792	flagellar M-ring protein
<i>fliG</i>	99.7	100	NP_249793	flagellar motor switch protein G
<i>fliH</i>	98.39	100	NP_249794	flagellar assembly protein H
<i>fliI</i>	99.26	100	NP_249795	flagellum-specific ATP synthase
<i>fliJ</i>	99.78	100	NP_249796	flagellar protein
<i>fliK</i>	97.2	100	NP_250132	flagellar hook-length control protein
<i>fliL</i>	99.62	100	NP_250133	flagellar basal body protein
<i>fliM</i>	99.9	100	NP_250134	flagellar motor switch protein
<i>fliN</i>	99.58	100	NP_250135	flagellar motor switch protein
<i>fliO</i>	99.56	100	NP_250136	flagellar protein FliO
<i>fliP</i>	99.09	100	NP_250137	flagellar biosynthetic protein
<i>fliQ</i>	99.63	100	NP_250138	flagellar biosynthetic protein
<i>fliR</i>	98.58	99.36	NP_250139	flagellar biosynthetic protein
<i>fliS</i>	99.47	100	NP_249786	flagellar protein
<i>fptA</i>	98.84	100	NP_252911	Fe(III)-pyochelin receptor precursor
<i>hcp1</i>	100	100	NP_248775	type VI secretion system substrate
<i>hsiA1</i>	98.65	100	NP_248772	type VI secretion system hcp secretion island protein
<i>hsiB1/vipA</i>	100	100	NP_248773	type VI secretion system tubule-forming protein
<i>hsiC1/vipB</i>	99.6	100	NP_248774	type VI secretion system tubule-forming protein
<i>hsiE1</i>	99.05	100	NP_248776	type VI secretion system hcp secretion island protein interacting with HsiB1 to form a novel subcomplex of the T6SS
<i>hsiF1</i>	99.22	100	NP_248777	type VI secretion system hcp secretion island protein, a gp25-like protein but not exhibit lysozyme activity
<i>hsiG1</i>	99.03	100	NP_248778	type VI secretion system hcp secretion island protein
<i>hsiH1</i>	98.57	100	NP_248779	type VI secretion system hcp secretion island protein
<i>hsiJ1</i>	99.33	100	NP_248769	type VI secretion system hcp secretion island protein
<i>icmF1/tssM1</i>	98.91	100	NP_248767	type VI secretion system protein

<i>lasA</i>	97.85	100	NP_250562	LasA protease precursor
<i>lasB</i>	98.4	100	NP_252413	elastase
<i>lasI</i>	99.17	100	NP_250123	autoinducer synthesis protein
<i>lip1</i>	99.57	100	NP_248770	lipoprotein
<i>mbtH-like</i>	100	100	NP_251102	MbtH-like protein from the pyoverdine cluster
<i>motA</i>	97.89	100	NP_253641	flagellar motor protein [Deoxyhexose linking sugar 209 Da capping structure (A1138)]
<i>motB</i>	99.14	100	NP_253640	flagellar motor protein [Deoxyhexose linking sugar 209 Da capping structure (A1138)]
<i>motC</i>	98.65	100	NP_250151	flagellar motor protein [Deoxyhexose linking sugar 209 Da capping structure (A1138)]
<i>motD</i>	98.43	100	NP_250152	flagellar motor protein [Deoxyhexose linking sugar 209 Da capping structure (A1138)]
<i>motY</i>	99.59	100	NP_252216	probable outer membrane protein precursor [Deoxyhexose linking sugar 209 Da capping structure (A1138)]
<i>mucA</i>	99.14	100	NP_249454	alkaline metalloproteinase precursor [Alginate (VF0091)]
<i>mucB</i>	98.95	100	NP_249455	anti-sigma factor MucA inhibitor of alg gene expression
<i>mucC</i>	99.78	100	NP_249456	negative regulator for alginate biosynthesis MucB [Alginate (VF0091)]
<i>mucD</i>	98.88	100	NP_249457	serine protease MucD precursor [Alginate regulation (CVF523)]
<i>mucE</i>	98.15	100	NP_252722	small envelope protein
<i>mucP</i>	99.04	100	NP_252339	metalloprotease protease
<i>pchA</i>	98.95	100	NP_252921	salicylate biosynthesis isochorismate synthase
<i>pchB</i>	99.67	100	NP_252920	salicylate biosynthesis protein
<i>pchC</i>	99.34	100	NP_252919	pyochelin biosynthetic protein
<i>pchD</i>	98.78	100	NP_252918	pyochelin biosynthesis protein
<i>pchE</i>	98.54	100	NP_252916	dihydroaeruginic acid synthetase
<i>pchF</i>	98.45	100	NP_252915	pyochelin synthetase
<i>pchG</i>	99.14	100	NP_252914	pyochelin biosynthetic protein
<i>pchH</i>	98.66	100	NP_252913	ABC transporter ATP-binding protein [
<i>pchI</i>	98.9	100	NP_252912	ABC transporter ATP-binding protein [Pyochelin
<i>pchR</i>	98.99	100	NP_252917	transcriptional regulator
<i>pcr1</i>	98.92	100	NP_250390	type III secretion system protein
<i>pcr2</i>	98.92	100	NP_250391	type III secretion system protein
<i>pcr3</i>	98.36	100	NP_250392	type III secretion system protein
<i>pcr4</i>	98.79	100	NP_250393	type III secretion system protein
<i>pcrD</i>	98.96	100	NP_250394	type III secretion system protein
<i>pcrG</i>	99.66	100	NP_250396	type III secretion system cytoplasmic regulator

<i>pcrH</i>	99.21	100	NP_250398	type III secretion system regulatory protein
<i>pcrR</i>	99.31	100	NP_250395	type III secretion system regulatory protein
<i>pcrV</i>	98.98	100	NP_250397	type III secretion system hydrophilic translocator needle tip protein
<i>phzA1</i>	96.16	90.59	NP_252899	phenazine biosynthesis protein
<i>phzA1</i>	99.39	100	NP_252899	phenazine biosynthesis protein
<i>phzB1</i>	98.36	100	NP_252900	phenazine biosynthesis protein
<i>phzC1</i>	99.18	100	NP_252901	phenazine biosynthesis protein
<i>phzD1</i>	98.88	100	NP_252902	phenazine biosynthesis protein
<i>phzE1</i>	99.1	100	NP_252903	phenazine biosynthesis protein
<i>phzF1</i>	99.16	100	NP_252904	phenazine biosynthesis protein
<i>phzG1</i>	99.53	100	NP_252906	phenazine biosynthesis protein
<i>phzH</i>	98.91	100	NP_248741	phenazine-modifying enzyme
<i>phzM</i>	99.4	100	NP_252898	phenazine-specific methyltransferase
<i>phzS</i>	98.35	100	NP_252907	flavin dependent hydroxylase
<i>pilB</i>	99	100	NP_253216	type 4 fimbrial biogenesis protein
<i>pilE</i>	99.06	100	NP_253246	type 4 fimbrial biogenesis protein
<i>pilF</i>	98.95	100	NP_252494	type 4 fimbrial biogenesis protein
<i>pilG</i>	100	100	NP_249099	twitching motility protein
<i>pilH</i>	99.18	100	NP_249100	twitching motility protein
<i>pilI</i>	100	100	NP_249101	twitching motility protein
<i>pilJ</i>	99.51	100	NP_249102	twitching motility protein
<i>pilK</i>	99.2	100	NP_249103	methyltransferase PilK
<i>pilM</i>	99.81	100	NP_253731	type IV pilus inner membrane platform protein
<i>pilN</i>	99.5	100	NP_253730	type IV pilus inner membrane platform protein
<i>pilO</i>	99.52	100	NP_253729	type IV pilus inner membrane platform protein
<i>pilP</i>	99.24	100	NP_253728	type IV pilus biogenesis protein
<i>pilQ</i>	99.07	100	NP_253727	type 4 fimbrial biogenesis protein
<i>pilR</i>	99.18	100	NP_253237	two-component response regulator PilR
<i>pilS</i>	99.18	100	NP_253236	two-component sensor
<i>pilT</i>	99.61	100	NP_249086	twitching motility protein
<i>pilU</i>	99.74	100	NP_249087	twitching motility protein
<i>pilV</i>	99.1	100	NP_253241	type IV pilus biogenesis protein
<i>pilW</i>	98.91	100	NP_253242	type IV fimbrial biogenesis protein
<i>pilX</i>	99.32	100	NP_253243	type 4 fimbrial biogenesis protein
<i>pilY1</i>	93.35	99.43	NP_253244	type 4 fimbrial biogenesis protein
<i>pilY2</i>	96.26	100	NP_253245	type 4 fimbrial biogenesis protein
<i>plcH</i>	96.08	100	NP_249535	hemolytic phospholipase C precursor

<i>popB</i>	99.15	100	NP_250399	type III secretion system hydrophobic translocator pore protein
<i>popD</i>	97.41	100	NP_250400	type III secretion system hydrophobic translocator pore protein
<i>popN</i>	99.42	100	NP_250389	type III secretion system outer membrane protein
<i>ppkA</i>	99.23	100	NP_248764	serine/threonine protein kinase
<i>pppA</i>	99.45	100	NP_248765	Pseudomonas protein phosphatase
<i>pscB</i>	99.05	100	NP_250406	type III secretion system protein
<i>pscC</i>	99.5	100	NP_250407	type III secretion system secretin
<i>pscD</i>	99	100	NP_250408	type III secretion system basal body protein
<i>pscE</i>	97.06	100	NP_250409	type III secretion system cochaperone
<i>pscF</i>	99.22	100	NP_250410	type III secretion system needle filament protein
<i>pscG</i>	99.71	100	NP_250411	type III secretion system chaperone PscG
<i>pscH</i>	100	100	NP_250412	type III secretion system protein PscH
<i>pscI</i>	99.11	100	NP_250413	type III secretion system inner rod protein PscI
<i>pscJ</i>	99.33	100	NP_250414	type III secretion system inner MS ring protein
<i>pscK</i>	98.09	99.04	NP_250415	type III secretion system protein
<i>pscL</i>	98.61	100	NP_250416	type III secretion system protein
<i>pscN</i>	99.24	100	NP_250388	type III secretion system ATPase
<i>pscO</i>	99.37	100	NP_250387	type III secretion system protein
<i>pscP</i>	94.64	96.49	NP_250386	type III secretion system protein
<i>pscQ</i>	98.82	100	NP_250385	type III secretion system protein
<i>pscR</i>	99.85	100	NP_250384	type III secretion system protein
<i>pscS</i>	99.25	100	NP_250383	type III secretion system protein
<i>pscT</i>	99.24	100	NP_250382	type III secretion system protein
<i>pscU</i>	98.76	100	NP_250381	type III secretion system protein
<i>ptxR</i>	98.08	99.79	NP_250948	transcriptional regulator
<i>pvcA</i>	98.68	100	NP_250944	paerucumarin biosynthesis protein
<i>pvcB</i>	96.98	100	NP_250945	paerucumarin biosynthesis protein
<i>pvcC</i>	99.27	100	NP_250946	paerucumarin biosynthesis protein
<i>pvcD</i>	97.99	99.85	NP_250947	paerucumarin biosynthesis protein
<i>pvdG</i>	98.69	100	NP_251115	pyoverdine biosynthesis protein
<i>pvdH</i>	99.15	100	NP_251103	diaminobutyrate-2-oxoglutarate aminotransferase
<i>pvdL</i>	98.8	100	NP_251114	peptide synthase [pyoverdine (IA001)]
<i>pvdM</i>	94.44	99.78	NP_251083	dipeptidase precursor [pyoverdine (IA001)] [Pseudomonas aeruginosa PAO1]
<i>pvdN</i>	99.45	100	NP_251084	pyoverdine biosynthesis protein
<i>pvdO</i>	97.78	100	NP_251085	pyoverdine biosynthesis protein
<i>pvdQ</i>	99.13	100	NP_251075	3-oxo-C12-homoserine lactone acylase

<i>pvdS</i>	97.34	100	NP_251116	extracytoplasmic-function sigma-70 factor
<i>rhlA</i>	99.1	100	NP_252169	rhamnosyltransferase chain A
<i>rhlB</i>	99.61	100	NP_252168	rhamnosyltransferase chain B
<i>rhlC</i>	97.03	100	NP_249821	rhamnosyltransferase 2
<i>rhlI</i>	100	100	NP_252166	autoinducer synthesis protein RhlI [Quorum sensing]
<i>tagF/pppB</i>	98.68	100	NP_248766	Pseudomonas protein phosphatase
<i>tagQ</i>	98.25	100	NP_248760	type VI secretion associated protein
<i>tagR</i>	98.95	100	NP_248761	type IV secretion associated protein
<i>tagS</i>	99.25	100	NP_248762	type IV secretion associated protein
<i>tagT</i>	98.89	100	NP_248763	type six secretion associated protein
<i>toxA</i>	99.01	100	NP_249839	exotoxin A precursor
<i>tse1</i>	99.57	100	NP_250535	type VI secretion system effector Tse1 peptidoglycanhydrolase
<i>tse2</i>	98.53	100	NP_251392	type VI secretion system effector Tse2 [HSI-1 (Hcp-secretion island 1)]
<i>tse3</i>	98.94	100	NP_252174	type VI secretion system effector Tse3 glycoside hydrolase
<i>vgrG1a</i>	98.45	100	NP_248781	type VI secretion system substrate
<i>waaA</i>	99.53	100	NP_253675	lipopolysaccharide core biosynthesis protein WaaP
<i>waaC</i>	98.88	100	NP_253698	3-deoxy-D-manno-octulosonic-acid (KDO) transferase
<i>waaF</i>	99.61	100	NP_253699	heptosyltransferase I
<i>waaG</i>	99.2	100	NP_253697	B-band O-antigen polymerase
<i>waaP</i>	99.5	100	NP_253696	UDP-glucose:(heptosyl) LPS alpha 13-glucosyltransferase
<i>xcpA/pilD</i>	99.65	99.31	NP_253218	type 4 prepilin peptidase PilD [Type IV pili]
<i>xcpP</i>	98.16	100	NP_251794	secretion protein
<i>xcpQ</i>	97.62	100	NP_251795	general secretion pathway protein D [xcp secretion system (VF0084)]
<i>xcpR</i>	98.01	100	NP_251793	general secretion pathway protein E [xcp secretion system (VF0084)]
<i>xcpS</i>	99.02	100	NP_251792	general secretion pathway protein F [xcp secretion system (VF0084)]
<i>xcpT</i>	99.11	100	NP_251791	general secretion pathway protein G [xcp secretion system (VF0084)]
<i>xcpU</i>	98.65	100	NP_251790	general secretion pathway protein H [xcp secretion system (VF0084)]
<i>xcpV</i>	98.97	99.49	NP_251789	general secretion pathway protein I [xcp secretion system (VF0084)]
<i>xcpW</i>	98.88	100	NP_251788	general secretion pathway protein J [xcp secretion system (VF0084)]
<i>xcpX</i>	96.51	100	NP_251787	general secretion pathway protein K [xcp secretion system (VF0084)]
<i>xcpY</i>	98.87	100	NP_251786	general secretion pathway protein L [xcp secretion system (VF0084)]
<i>xcpZ</i>	99.24	100	NP_251785	general secretion pathway protein M [xcp secretion system (VF0084)]
<i>phzB1</i>	89.89	88.96	NP_252900	phenazine biosynthesis protein
<i>pilC</i>	76.17	98.31	NP_253217	still frameshift type 4 fimbrial biogenesis protein
<i>pvdA</i>	83.56	100	NP_251076	L-ornithine N5-oxygenase PvdA
<i>pvdF</i>	82.49	100	NP_251086	pyoverdine synthetase F
<i>pvdP</i>	85.04	99.76	NP_251082	tyrosinase required for pyoverdine maturation

Table S7 Antibiotic resistance genes detected in *Pseudomonas aeruginosa* Castle Beach clone.

<i>APH(3')-IIb</i>	98.64	100	X90856:388-1195	a chromosomal-encoded aminoglycoside phosphotransferase
<i>ArmR</i>	96.3	100	NC_002516.2:4165719-4165881	a 53-amino-acid antirepressor allosterically inhibits MexR dimer-DNA binding by occupying a hydrophobic binding cavity within the center of the MexR dimer. ArmR up-regulation and MexR-ArmR complex formation have previously been shown to upregulate MexAB-OprM.
<i>arnA</i>	98.99	100	NC_002516.2:3982021-3984010	modifies lipid A with 4-amino-4-deoxy-L-arabinose (Ara4N) which allows gram-negative bacteria to resist the antimicrobial activity of cationic antimicrobial peptides and antibiotics such as polymyxin.
<i>basS</i>	98.75	100	JQ340365:1-1435	Histidine protein kinase sensor Lipid A modification gene; part of a two-component system involved in polymyxin resistance that senses high extracellular Fe(2+)
<i>bcr-1</i>	98.92	100	CP012901.1:5979157-5980366	Transmembrane protein which expels bicyclomycin from the cell leading to bicyclomycin resistance. Identified in <i>P. aeruginosa</i> strains responsible for outbreaks in Brazil often appearing with blaSPM-1 another bicyclomycin resistance gene
<i>catB7</i>	97.66	100	NC_002516:779463-780102	chromosome-encoded variant of the cat gene
<i>fosA</i>	99.51	100	NC002516:1221691-1222099	enzyme that confers resistance to fosfomycin in <i>Serratia marcescens</i> by breaking the epoxide ring of the molecule. It depends on the cofactors Manganese (II) and Potassium and uses Glutathione (GSH) as the nucleophilic molecule. In <i>P. aeruginosa</i> FosA catalyzes the conjugation of glutathione to carbon-1 of fosfomycin rendering it ineffective as an antibacterial drug.
<i>MexA</i>	99.31	100	NC_002516:472024-473176	membrane fusion protein of the MexAB-OprM multidrug efflux complex
<i>MexB</i>	99.01	100	L11616:1570-4711	inner membrane multidrug exporter of the efflux complex MexAB-OprM

<i>MexC</i>	99.23	100	U57969:295-1459	membrane fusion protein of the MexCD-OprJ multidrug efflux complex
<i>MexD</i>	97.73	99.94	U57969:1486-4618	multidrug inner membrane transporter of the MexCD-OprJ complex
<i>MexE</i>	99.12	100	NC_002516:2808743-2809988	membrane fusion protein of the MexEF-OprN multidrug efflux complex
<i>MexF</i>	99.28	100	NC_002516:2810009-2813198	multidrug inner membrane transporter of the MexEF-OprN complex. <i>mexF</i> corresponds to 2 loci in <i>P. aeruginosa</i> PAO1 (<i>mexF/mexB</i>) and 4 loci in <i>P. aeruginosa</i> LESB58 (<i>mexD/mexB</i>).
<i>mexG</i>	99.78	100	NC_002516:4705956-4706403	membrane protein required for MexGHI-OpmD efflux activity
<i>mexH</i>	99.1	100	NC_002516:4706410-4707523	membrane fusion protein of the efflux complex MexGHI-OpmD
<i>mexI</i>	99.39	100	NC_002516:4707535-4710625	inner membrane transporter of the efflux complex MexGHI-OpmD
<i>mexJ</i>	98.91	100	NC_002516.2:4119270-4120374	membrane fusion protein of the MexJK multidrug efflux protein
<i>mexK</i>	98.7	100	AE004091.2:4116188-4119266	inner membrane resistance-nodulation-cell division (RND) transporter in the MexJK multidrug efflux protein
<i>mexL</i>	99.53	100	AE004091.2:4120469-4121108	a specific repressor of <i>mexJK</i> transcription and autoregulates its own expression
<i>mexM</i>	99.31	100	AB219523.1:22-1180	membrane fusion protein of the MexMN-OprM multidrug efflux complex
<i>mexN</i>	99.29	100	AB219523.1:1176-4287	inner membrane transporter of the MexMN-OprM multidrug efflux complex
<i>mexP</i>	98.88	100	AB219524.1:23-1181	membrane fusion protein of the MexPQ-OpmE multidrug efflux complex
<i>mexQ</i>	98.89	100	AB219524.1:1177-4339	inner membrane transporter of the multidrug efflux pump MexPQ-OpmE
<i>mexV</i>	98.67	100	AE004091.2:4903466-4904597	membrane fusion protein of the MexVW-OprM multidrug efflux complex
<i>mexW</i>	99.48	100	NC_002516.2:4904647-4907704	RND-type membrane protein of the efflux complex MexVW-OprM
<i>mexX</i>	97.71	100	AB015853:146-1316	membrane fusion protein of the MexXY-OprM multidrug efflux complex
<i>mexY</i>	98.31	99.81	AB015853:1331-4472	RND-type membrane protein of the efflux complex MexXY-OprM
<i>MuxA</i>	98.98	100	NC_002516.2:2854011-2855292	membrane fusion protein component of the efflux pump system MuxABC-OpmB in <i>P. aeruginosa</i>

<i>MuxB</i>	99.33	100	NC_002516.2:2850883-2854015	one of the two necessary RND components in the <i>P. aeruginosa</i> efflux pump system MuxABC-OpmB.
<i>MuxC</i>	98.94	100	NC_002516.2:2847776-2850887	one of the two necessary RND components of the MuxABC-OpmB efflux pumps system in <i>P. aeruginosa</i>
<i>OpmB</i>	99.13	100	NC_002516.2:2846283-2847780	outer membrane efflux protein in <i>P. aeruginosa</i> that shows functional cooperation with MuxABC to form the efflux pump system MuxABC-OpmB.
<i>opmD</i>	98.57	99.93	NC_002516:4710621-4712085	outer membrane channel protein of the efflux complex MexGHI-OpmD.
<i>opmE</i>	99.25	100	AB219524.1:4335-5811	outer membrane factor protein that is part of the multidrug efflux pump MexPQ-OpmE.
<i>OpmH</i>	99.45	100	NC_002516.2:5584101-5585550	outer membrane efflux protein required for triclosan-specific efflux pump function.
<i>OprJ</i>	98.61	100	U57969:4623-6063	outer membrane channel component of the MexCD-OprJ multidrug efflux complex.
<i>OprM</i>	99.18	100	NC_002516:476333-477791	outer membrane factor protein found in <i>P. aeruginosa</i> and <i>Burkholderia vietnamiensis</i> . It is part of the MexAB-OprM MexVW-OprM MexXY-OprM and the AmrAB-OprM complex.
<i>OprN</i>	98.45	100	NC_002516:2813194-2814613	outer membrane channel component of the MexEF-OprN multidrug efflux complex.
<i>OXA-50</i>	98.23	100	AY306130:1-790	beta-lactamase found in <i>P. aeruginosa</i> . It confers decreased susceptibility to ampicillin and ticarcillin and interestingly to moxalactam and meropenem in <i>P. aeruginosa</i> but not in <i>E. coli</i> . Also confers resistance to piperacillin-tazobactam and cephalotin.
<i>PDC-5</i>	99.41	100	FJ666068:1-1195	extended-spectrum beta-lactamase found in <i>P. aeruginosa</i>
<i>PmpM</i>	99.16	100	NC_002516.2:1472547-1473981	multidrug efflux pump belonging to the MATE family of <i>P. aeruginosa</i> . PmpM is an H ⁺ drug antiporter and is the first reported case of an H ⁺ coupled efflux pump in the MATE family. PmpM confers resistance to fluoroquinolones fradiomycin benzalkonium chloride chlorhexidine gluconate ethidium bromide tetraphenylphosphonium chloride (TPPCl) and rhodamine 6G
<i>Pseudomonas_aeruginosa_CpxR</i>	99.56	100	LT673656.1:1884345-1885023	directly involved in activation of expression of RND efflux pump MexAB-OprM in <i>P. aeruginosa</i> . CpxR is required to enhance mexAB-oprM expression and drug resistance in the absence of repressor MexR.

<i>Pseudomonas_aeruginosa_emrE</i>	99.1	100	NC_002516.2:1-334	a small multidrug transporter that functions as a homodimer and that couples the efflux of small polyaromatic cations from the cell with the import of protons down an electrochemical gradient. Confers resistance to tetraphenylphosphonium methyl viologen gentamicin kanamycin and neomycin.
<i>Pseudomonas_aeruginosa_soxR</i>	98.94	100	NC_002516.2:2503425-2503896	SoxR is a redox-sensitive transcriptional activator that induces expression of a small regulon that includes the RND efflux pump-encoding operon mexGHI-opmD. SoxR was shown to be activated by pyocyanin.
<i>TriA</i>	99.22	100	NC_002516.2:177307-178459	membrane protein that is fused to TriB and both are required for the triclosan efflux pump function of TriABC-OpmH in <i>P. aeruginosa</i>
<i>TriB</i>	99.25	100	NC_002516.2:178455-179526	membrane protein that is fused to TriA and both are required for the triclosan efflux pump function of TriABC-OpmH in <i>P. aeruginosa</i>
<i>TriC</i>	99.18	100	NC_002516.2:179522-182570	resistance nodulation cell division (RND) transporter that is a part of TriABC-OpmH a triclosan-specific efflux protein.
<i>AxyY</i>	78.44	97.74	AFRQ01000061.1:23987-27125	periplasmic adaptor protein of the AxyXY-OprZ efflux pump system in <i>Achromobacter spp.</i>

Table S8 Virulence genes detected in *Escherichia coli* Mylor Harbour.

gene name	nt identity (%)	coverage (%)	acc. nr.	description
<i>aslA</i>	98.25	100	AAG10151	putative arylsulfatase
<i>chuA</i>	99.74	100	NP_756170	Outer membrane heme/hemoglobin receptor
<i>chuS</i>	99.51	100	NP_756169	heme oxygenase
<i>chuT</i>	99.5	100	NP_756175	periplasmic heme-binding protein
<i>chuU</i>	99.09	100	NP_756179	heme permease protein
<i>chuV</i>	98.38	100	NP_756180	ATP-binding hydrophilic protein
<i>chuW</i>	99.33	100	NP_756176	Putative oxygen independent coproporphyrinogen III oxidase
<i>chuX</i>	100	100	NP_756177	putative heme-binding protein
<i>chuY</i>	100	100	NP_756178	ChuY
<i>entA</i>	98.26	100	NP_752614	23-dihydro-23-dihydroxybenzoate dehydrogenase [Enterobactin (VF0228)]
<i>entB</i>	99.77	100	NP_752613	isochorismatase [Enterobactin (VF0228)]
<i>entC</i>	98.57	100	NP_752611	isochorismate synthase 1 [Enterobactin (VF0228)]

<i>entD</i>	99.87	100	NP_752599	phosphopantetheinyl transferase component of enterobactin synthase multienzyme complex [Enterobactin (VF0228)]
<i>entE</i>	98.39	100	NP_752612	23-dihydroxybenzoate-AMP ligase component of enterobactin synthase multienzyme complex [Enterobactin (VF0228)]
<i>entF</i>	98.7	99.28	NP_752604	enterobactin synthase multienzyme complex component ATP-dependent [Enterobactin (VF0228)]
<i>entS</i>	98.32	100	NP_752609	enterobactin exporter iron-regulated [enterobactin (IA019)]
<i>fdeC</i>	98.28	100	YP_002390132	adhesin
<i>fepA</i>	99.06	100	NP_752600	ferrienterobactin outer membrane transporter [Enterobactin (VF0228)]
<i>fepB</i>	99.48	100	NP_752610	ferrienterobactin ABC transporter periplasmic binding protein [Enterobactin (VF0228)]
<i>fepC</i>	98.9	100	NP_752606	ferrienterobactin ABC transporter ATPase [Enterobactin (VF0228)]
<i>fepD</i>	98.92	100	NP_752608	ferrienterobactin ABC transporter permease [Enterobactin (VF0228)]
<i>fepG</i>	98.49	100	NP_752607	iron-enterobactin ABC transporter permease [Enterobactin (VF0228)]
<i>fes</i>	99.58	100	NP_752602	enterobactin/ferric enterobactin esterase [Enterobactin (IA019)]
<i>fimA</i>	92.08	100	NP_757241	Type-1 fimbrial protein A chain precursor
<i>fimB</i>	99.83	100	NP_757239	Type 1 fimbriae Regulatory protein
<i>fimC</i>	99.04	100	NP_757243	Chaperone protein fimC precursor
<i>fimD</i>	99.01	100	NP_757244	Outer membrane usher protein fimD precursor
<i>fimE</i>	100	100	NP_757240	Type 1 fimbriae Regulatory protein
<i>fimF</i>	99.81	100	NP_757245	FimF protein precursor
<i>fimG</i>	98.81	100	NP_757247	FimG protein precursor
<i>fimH</i>	98.9	100	NP_757248	FimH protein precursor
<i>fimI</i>	99.44	100	NP_757242	Fimbrin-like protein fimI precursor
<i>fyuA</i>	99.8	100	NP_405467	pesticin/yersiniabactin receptor protein
<i>gspM</i>	92.62	92.91	YP_404609	general secretion pathway protein M
<i>ibeA</i>	98.47	99.93	AAF98391	invasion protein
<i>irp1</i>	99.69	100	NP_405471	yersiniabactin biosynthetic protein
<i>irp2</i>	99.44	100	NP_405472	yersiniabactin biosynthetic protein
<i>ompA</i>	99.81	100	AAF37887	outer membrane protein A
<i>set1A</i>	94.38	99.44	YP_006098866	toxin subunit
<i>set1B</i>	98.39	100	YP_006098865	toxin subunit
<i>vat</i>	99.9	100	NP_752330	Haemoglobin protease
<i>yagV/ecpE</i>	98.14	99.74	NP_286006	<i>E. coli</i> common pilus chaperone
<i>yagW/ecpD</i>	99.09	100	NP_286007	polymerized tip adhesin of ECP fibers
<i>yagX/ecpC</i>	97.78	100	NP_286008	<i>E. coli</i> common pilus usher
<i>yagY/ecpB</i>	97.76	100	NP_286009	<i>E. coli</i> common pilus chaperone

<i>yagZ/ecpA</i>	98.3	100	NP_286010	<i>E. coli</i> common pilus structural subunit
<i>ybtA</i>	99.58	100	NP_405473	transcriptional regulator
<i>ybtE</i>	99.94	100	NP_405468	yersiniabactin siderophore biosynthetic protein
<i>ybtP</i>	99.61	100	NP_405474	lipoprotein inner membrane ABC-transporter
<i>ybtQ</i>	99.72	100	NP_405475	inner membrane ABC-transporter
<i>ybtS</i>	99.69	100	NP_405477	salicylate synthase Irp9
<i>ybtT</i>	99.75	100	NP_405469	yersiniabactin biosynthetic protein
<i>ybtU</i>	99.73	100	NP_405470	yersiniabactin biosynthetic protein
<i>ybtX</i>	99.69	100	NP_405476	putative signal transducer
<i>ykgK/ecpR</i>	99.32	100	NP_286011	regulator protein
<i>cheW</i>	75.11	90.36	YP_001006779	purine-binding chemotaxis protein
<i>cheY</i>	77.06	99.49	YP_001006774	chemotaxis regulatory protein
<i>csgB</i>	83.59	99.78	NP_460114	minor curlin subunit precursor curli nucleator protein
<i>csgD</i>	81.41	100	NP_460113	DNA-binding transcriptional regulator
<i>csgE</i>	79.29	98.48	NP_460112	curli production assembly/transport protein
<i>csgF</i>	81	99.04	NP_460111	curli production assembly/transport protein
<i>csgG</i>	83.45	100	NP_460110	curli production assembly/transport protein
<i>flgG</i>	75.81	98.93	YP_001006759	flagellar basal-body rod protein
<i>flgH</i>	78.99	80.7	YP_001006758	flagellar L-ring protein precursor
<i>flhA</i>	75.01	99.04	YP_001006770	flagellar biosynthesis protein
<i>flhC</i>	75.05	95.19	YP_001006783	flagellar biosynthesis transcription activator
<i>fliG</i>	78.54	99.5	YP_001006742	flagellar motor switch protein G
<i>fliI</i>	75.74	92.79	YP_001006744	flagellum-specific ATP synthase
<i>fliM</i>	76.33	99.5	YP_001006748	flagellar motor switch protein
<i>fliP</i>	77.43	98.4	YP_001006751	flagellar biosynthetic protein

Table S9 Antibiotic resistance genes detected in *Escherichia coli* Mylor Harbour.

gene name	nt identity (%)	coverage (%)	acc. nr.	description
<i>acrB</i>	98.83	100	NC_000913.3:481254-484404	Protein subunit of AcrA-AcrB-TolC multidrug efflux complex. <i>AcrB</i> functions as a heterotrimer which forms the inner membrane component and is primarily responsible for substrate recognition and energy transduction by acting as a drug/proton antiporter.
<i>acrD</i>	98.56	100	NC_007779:2586251-2589365	aminoglycoside efflux pump expressed in <i>E. coli</i> . Its expression can be induced by indole and is regulated by <i>baeRS</i> and <i>cpxABR</i> .
<i>acrE</i>	98.79	100	U00096:3413864-3415022	membrane fusion protein similar to AcrA.

<i>acrF</i>	96.59	100	U00096:3415033-3418138	inner membrane transporter similar to AcrB.
<i>acrS</i>	98.34	100	U00096:3412803-3413466	repressor of the AcrAB efflux complex and is associated with the expression of AcrEF. AcrS is believed to regulate a switch between AcrAB and AcrEF efflux.
<i>bacA</i>	98.17	99.76	U00096:3203310-3204132	gene that recycles undecaprenyl pyrophosphate during cell wall biosynthesis which confers resistance to bacitracin
<i>baeR</i>	97.23	99.72	NC_007779:2166413-2167136	response regulator that promotes the expression of MdtABC and AcrD efflux complexes.
<i>baeS</i>	90.46	100	AP009048:2165013-2166417	sensor kinase in the BaeSR regulatory system. While it phosphorylates BaeR to increase its activity BaeS is not necessary for overexpressed BaeR to confer resistance.
<i>cpxA</i>	98.47	99.56	NC_002695:4903562-4904936	a membrane-localized sensor kinase that is activated by envelope stress. It starts a kinase cascade that activates CpxR which promotes efflux complex expression.
<i>CRP</i>	99.05	100	AP009048:4153664-4154297	global regulator that represses MdtEF multidrug efflux pump expression.
<i>emrA</i>	98.13	100	AP009048:2810083-2811256	membrane fusion protein providing an efflux pathway with EmrB and TolC between the inner and outer membranes of <i>E. coli</i> .
<i>emrB</i>	97.99	100	U00096:2812616-2814155	translocase in the <i>emrB</i> -TolC efflux protein in <i>E. coli</i> . It recognizes substrates including carbonyl cyanide m-chlorophenylhydrazone (CCCP) nalidixic acid and thioactomycin.
<i>emrD</i>	97.82	100	GG749185.1:116813-118004	multidrug transporter from the Major Facilitator Superfamily (MFS) primarily found in <i>E. coli</i> . EmrD couples efflux of amphipathic compounds with proton import across the plasma membrane.
<i>emrK</i>	97.64	100	D78168:537-1593	membrane fusion protein that is a homolog of EmrA. Together with the inner membrane transporter EmrY and the outer membrane channel TolC it mediates multidrug efflux.
<i>emrR</i>	99.06	100	NC_000913.3:2810770-2811301	negative regulator for the EmrAB-TolC multidrug efflux pump in <i>E. coli</i> . Mutations lead to EmrAB-TolC overexpression.
<i>emrY</i>	97.66	100	D78168:1592-3131	multidrug transport that moves substrates across the inner membrane of the Gram-negative <i>E. coli</i> . It is a homolog of <i>emrB</i> .
<i>Escherichia_coli_acrA</i>	99.08	100	NC_000913.3:484426-485620	subunit of the AcrAB-TolC multidrug efflux system that in <i>E. coli</i> .
<i>Escherichia_coli_ampC</i>	98.06	100	NC_000913.3:4377811-4378945	class C ampC beta-lactamase (cephalosporinase) enzyme described in <i>E. coli</i> shown clinically to confer resistance to penicillin-like and cephalosporin-class antibiotics.
<i>Escherichia_coli_mdfA</i>	96.35	100	JQ394987:1-1234	Multidrug efflux pump in <i>E. coli</i> . This multidrug efflux system was originally identified as the Cmr/CmlA chloramphenicol exporter.

<i>evgA</i>	99.03	100	NC_002695:3211892-3212507	when phosphorylated is a positive regulator for efflux protein complexes emrKY and mdtEF. While usually phosphorylated in a EvgS dependent manner it can be phosphorylated in the absence of EvgS when overexpressed.
<i>evgS</i>	96.19	100	U00096:2484374-2487968	sensor protein that phosphorylates the regulatory protein EvgA. <i>evgS</i> corresponds to 1 locus in <i>P. aeruginosa</i> PAO1 and 1 locus in <i>P. aeruginosa</i> LESB58.
<i>gadW</i>	99.86	100	CP015085.1:2551712-2552441	AraC-family regulator that promotes mdtEF expression to confer multidrug resistance. GadW inhibits GadX-dependent activation. GadW clearly represses <i>gadX</i> and in situations where GadX is missing activates <i>gadA</i> and <i>gadBC</i> .
<i>gadX</i>	93.7	100	NC_007779:3974605-3975430	AraC-family regulator that promotes mdtEF expression to confer multidrug resistance.
<i>H-NS</i>	99.28	100	NC_002695:1737554-1737968	histone-like protein involved in global gene regulation in Gram-negative bacteria. It is a repressor of the membrane fusion protein genes <i>acrE</i> <i>mdtE</i> and <i>emrK</i> as well as nearby genes of many RND-type multidrug exporters.
<i>kdpE</i>	95.84	99.26	NC_000913.3:721056-721734	transcriptional activator that is part of the two-component system KdpD/KdpE that is studied for its regulatory role in potassium transport and has been identified as an adaptive regulator involved in the virulence and intracellular survival of pathogenic bacteria. <i>kdpE</i> regulates a range of virulence loci through direct promoter binding.
<i>marA</i>	98.18	100	NC_007779:1621288-1621672	In the presence of antibiotic stress <i>E. coli</i> overexpresses the global activator protein MarA which besides inducing MDR efflux pump AcrAB also down-regulates synthesis of the porin OmpF.
<i>mdtA</i>	95.84	100	U00096:2154016-2155264	membrane fusion protein of the multidrug efflux complex mdtABC.
<i>mdtB</i>	95.81	100	U00096:2155263-2158386	transporter that forms a heteromultimer complex with MdtC to form a multidrug transporter. MdtBC is part of the MdtABC-ToIC efflux complex.
<i>mdtC</i>	93.86	100	U00096:2158386-2161464	transporter that forms a heteromultimer complex with MdtB to form a multidrug transporter. MdtBC is part of the MdtABC-ToIC efflux complex. In the absence of MdtB MdtC can form a homomultimer complex that results in a functioning efflux complex with a narrower drug specificity. <i>mdtC</i> corresponds to 3 loci in <i>Pseudomonas aeruginosa</i> PAO1 (gene name: <i>muxC/muxB</i>) and 3 loci in <i>Pseudomonas aeruginosa</i> LESB58.
<i>mdtE</i>	98.53	100	AP009048:3980026-3981184	membrane fusion protein of the MdtEF multidrug efflux complex. It shares 70% sequence similarity with AcrA.
<i>mdtF</i>	97.24	100	U00096:3660414-3663528	multidrug inner membrane transporter for the MdtEF-ToIC efflux complex.

<i>mdtG</i>	98.37	100	NC_007779:1115841-1117068	also named YceE, appears to be a member of the major facilitator superfamily of transporters and it has been reported when overexpressed to increase fosfomycin and deoxycholate resistances. <i>mdtG</i> is a member of the <i>marA-soxS-rob</i> regulon.
<i>mdtH</i>	98.1	100	U00096:1124118-1125327	Multidrug resistance protein
<i>mdtN</i>	95.83	100	AP009048:4306557-4307589	Multidrug resistance efflux pump. Could be involved in resistance to puromycin acriflavine and tetraphenylarsonium chloride.
<i>mdtO</i>	97.17	100	AP009048:4304506-4306558	Multidrug resistance efflux pump. Could be involved in resistance to puromycin acriflavine and tetraphenylarsonium chloride
<i>mdtP</i>	97.48	100	AP009048:4303043-4304510	Multidrug resistance efflux pump. Could be involved in resistance to puromycin acriflavine and tetraphenylarsonium chloride
<i>msbA</i>	97.77	100	NC_000913.3:966621-968370	multidrug resistance transporter homolog from <i>E. coli</i> and belongs to a superfamily of transporters that contain an adenosine triphosphate (ATP) binding cassette (ABC) which is also called a nucleotide-binding domain (NBD). <i>MsbA</i> is a member of the MDR-ABC transporter group by sequence homology. <i>MsbA</i> transports lipid A a major component of the bacterial outer cell membrane and is the only bacterial ABC transporter that is essential for cell viability.
<i>patA</i>	97.03	100	NC_000913.3:3219494-3220874	<i>PatA</i> is an ABC transporter of <i>Streptococcus pneumoniae</i> that interacts with <i>PatB</i> to confer fluoroquinolone resistance.
<i>PmrC</i>	91.12	100	AP009048:4338625-4340269	mediates the modification of Lipid A by the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine resulting in a less negative cell membrane and decreased binding of polymyxin B.
<i>pmrE</i>	94.09	100	U00096:2098447-2099614	required for the synthesis and transfer of 4-amino-4-deoxy-L-arabinose (Ara4N) to Lipid A which allows gram-negative bacteria to resist the antimicrobial activity of cationic antimicrobial peptides and antibiotics such as polymyxin
<i>pmrF</i>	97.63	100	U00096:2367071-2368040	required for the synthesis and transfer of 4-amino-4-deoxy-L-arabinose (Ara4N) to Lipid A which allows gram-negative bacteria to resist the antimicrobial activity of cationic antimicrobial peptides and antibiotics such as polymyxin. <i>pmrF</i> corresponds to 1 locus in <i>Pseudomonas aeruginosa</i> PAO1 and 1 locus in <i>Pseudomonas aeruginosa</i> LESB58.
<i>tolC</i>	98.39	100	FJ768952:1-1489	<i>TolC</i> is a protein subunit of many multidrug efflux complexes in Gram negative bacteria. It is an outer membrane efflux protein and is constitutively open. Regulation of efflux activity is often at its periplasmic entrance by other components of the efflux complex.

<i>yojI</i>	97.57	99.94	NC_000913.3:1-1645	mediates resistance to the peptide antibiotic microcin J25 when it is expressed from a multicopy vector. YojI is capable of pumping out microcin molecules. The outer membrane protein TolC in addition to YojI is required for export of microcin J25 out of the cell. Microcin J25 is thus the first known substrate for YojI.
<i>mdtK</i>	75.27	98.74	CP014358.1:2161326-2162751	multidrug and toxic compound extrusions (MATE) transporter conferring resistance to norfloxacin doxorubicin and acriflavine.
<i>vgaC</i>	83.55	100	KU302801:102200-102431	efflux protein expressed in staphylococci that confers resistance to streptogramin A antibiotics and related compounds. It is associated with plasmid DNA.

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