Table S1: Summary information of the strains used in this study.

Strain	Genotype	Source isolated
Rd KW20	Serotype d, non - capsular	Lab strain
285	non-typeable (NTHi)	Otitis Media
86-028NP	NTHi	Otitis Media
86.0276MEE	NTHi	Otitis Media
C486	NTHI	Otitis Media
Eagan	Serotype b, capsular	Cerebral Serum Fluid
Hi667	NTHi	Otitis Media
R2846	NTHi	Otitis Media
R2866	NTHi	Blood
R3264	NTHi	Middle ear of Healthy Child
165	NTHi	Otitis Media

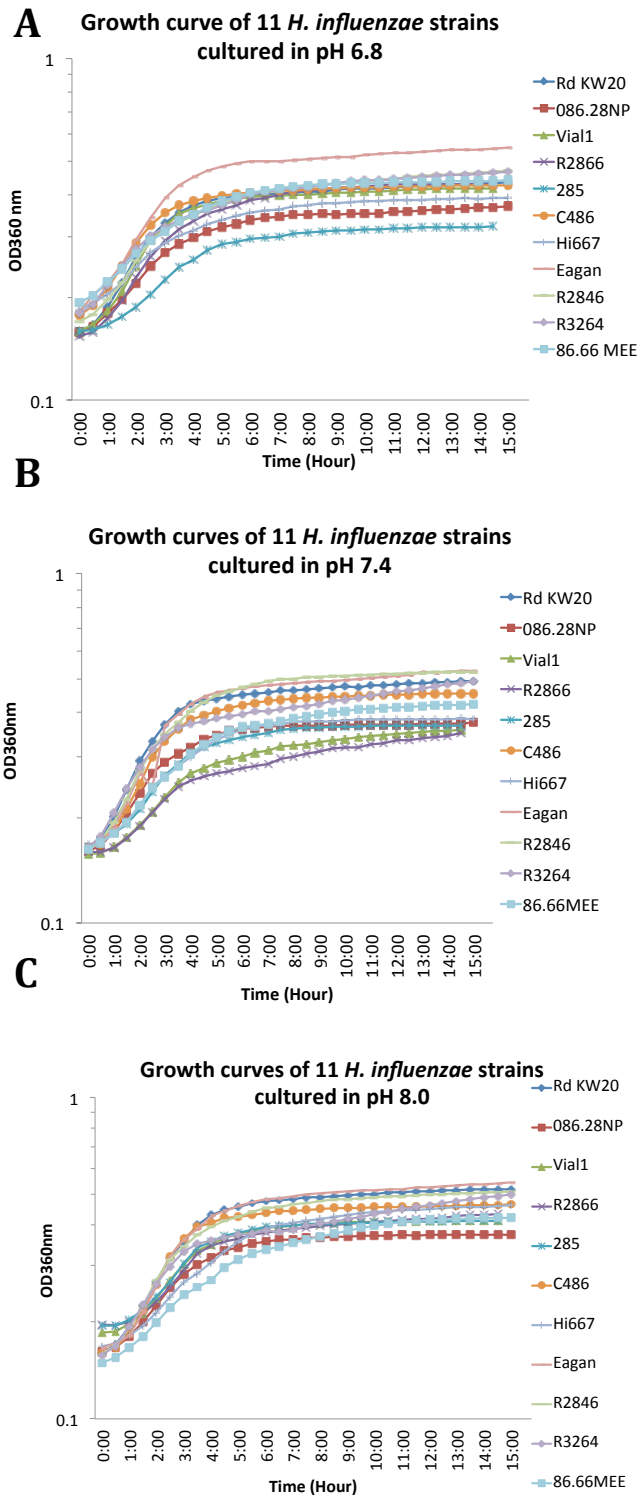

Figure S1: The growth profile of different strains of *H. influenzae* grown under different pH. The *H. influenzae* isolates as indicated were grown in HI broth pre-culture before a normalized amount inoculated into media at (A) pH 6.8, (B) pH 7.4 and (C) pH 8.0.

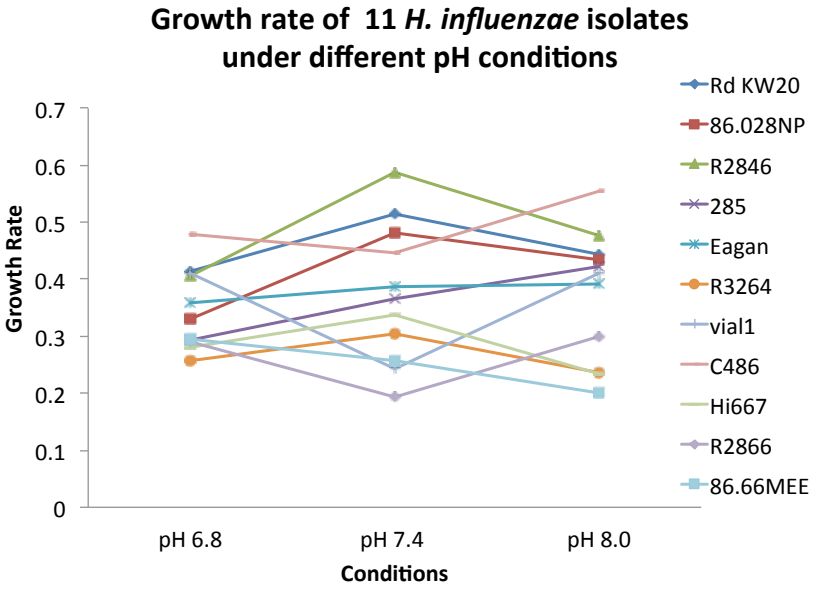

Figure S2: The growth rates of *H. influenzae* strains under different pH. The growth rates were calculated from Fig S1 and plotted.

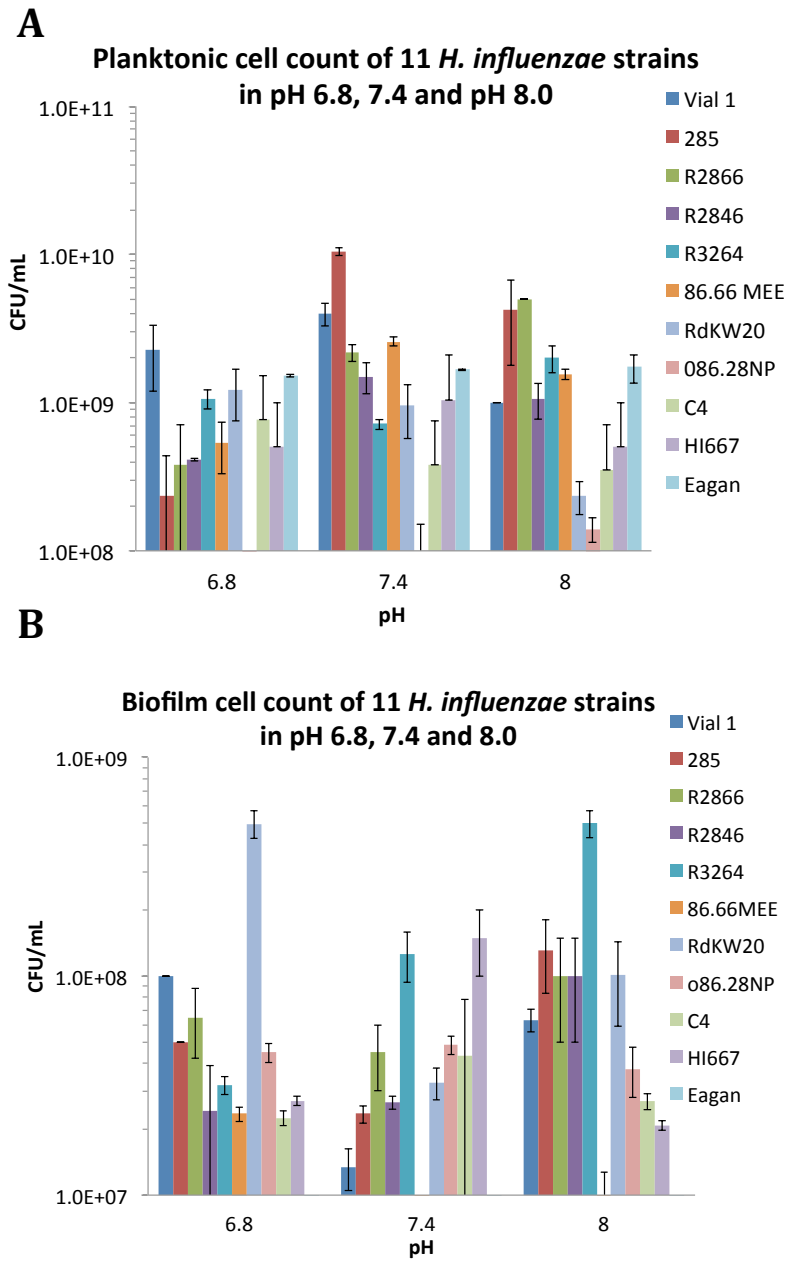

Figure S3: Viable cell counts of different *H. influenzae* strains grown under pH 6.8, 7.4 and 8.0. The isolates were grown and viable cells counted for the (A) planktonic cell number (CFU/ml) and concurrently the (B) biofilm cell number.

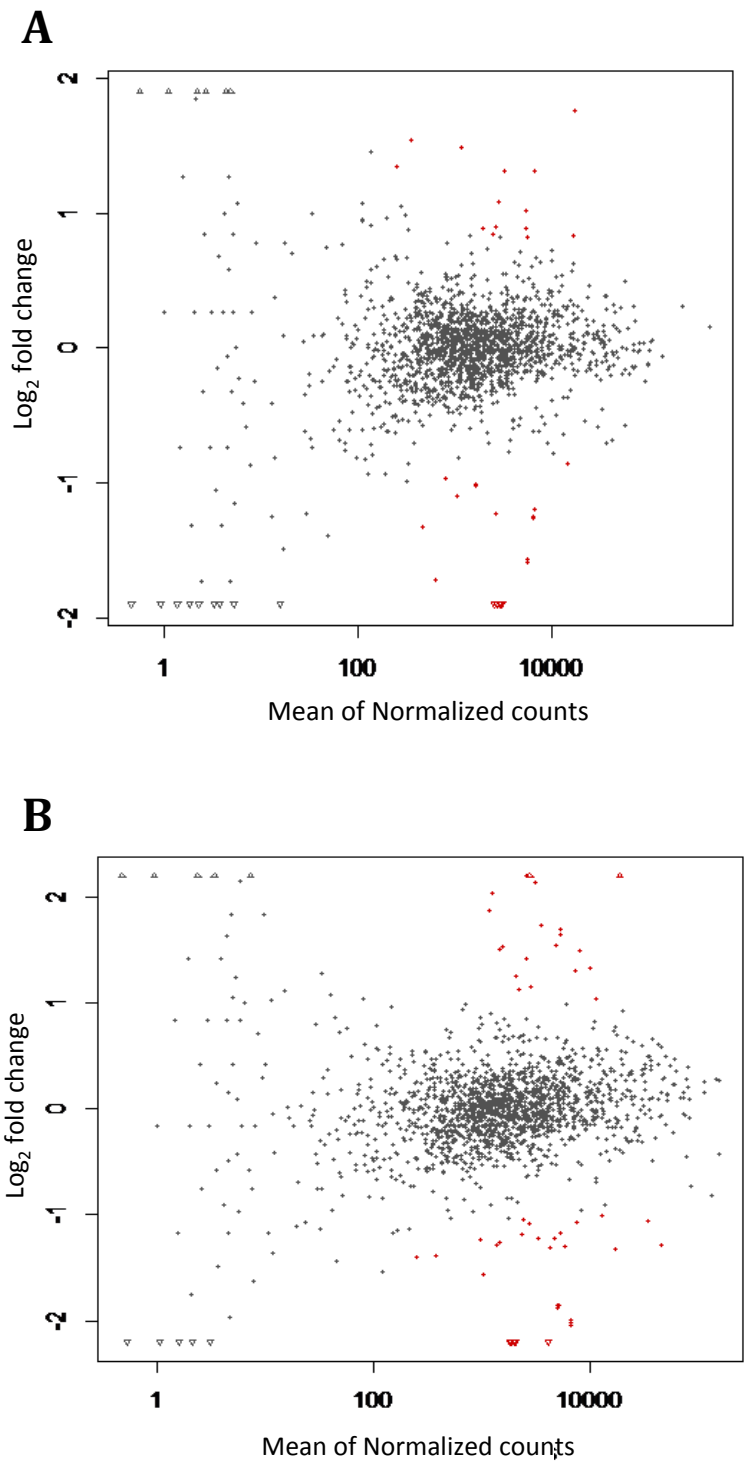

Figure S4: Scatter plots of log_2 fold change against normalized counts for each of the genes identified from mRNAseq. Each dot represents a gene identified from the RNA sequencing library and then compared for counts in the library from pH 8.0 compared to pH 6.8 for (A) R3264 and then for (B) Eagan. The dots that red are those genes determined as being significantly differentially expressed with p-value<0.05 and FDR<10%.