Title: Regarding flagellar expression in clinical isolates of non-typeable *Haemophilus influenzae*

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Haemophilus influenzae (HI) has historically been considered a non-motile, non-flagellate bacterium. It was, therefore, of considerable interest that Carabarin-Lima *et al.* [1] described “swarming motility” in numerous HI strains, and the presence of two flagella genes in strain BUAP96. One of the strains reported as motile was Rd KW20 (Rd), the first bacterium to have its whole genome sequenced [2], which does not possess any known flagella genes.

The authors used "degenerate" primers based on known enteric genes, to amplify weak bands of 1490 (*fliC*) and 700 (*flgH*) bp from BUAP96, plus an uncommented on circa 850 bp (*flgH*) band from Rd (Fig 2). We were unable to predict, by virtual PCR, any amplicon using the Rd whole genome sequence (accession number: L42023) and the primers stated. The sequenced 5’ and 3’ ends of BUAP96 *fliC* were reported to share “high homology” with known enteric flagellin genes, as well as an *Actinobacillus pleuropneumoniae* (APP) *fliC*-like gene [3], which has not been substantiated. However, no sequence data for BUAP96 *fliC* (or *flgH*) was presented, or made publicly available.

Carabarin-Lima *et al.* [1] further reported that flagellin-specific antisera, previously used to detect *Escherichia coli* flagellin and a similar sized protein from APP strain BC5235 [3], also recognized a similar protein from BUAP96 in Western blots (Fig 6b and 6c). The “anti-APP flagellin” serum used in both studies was raised against a BC5235 protein reportedly containing an N-terminal sequence (AQVINTNSLSLI) identical to that of known enteric FliC proteins [3]. Similar proteins were also recognised in Western blots using extracts of 12 APP serovar reference strains, including 5b L20 [3]. However, the complete genome sequence of L20 [4] does not contain *fliC, flgH,* or any known flagella genes. Indeed, no *fliC* or *flgH* homologues are present in any of the publicly available HI (or APP) genomes. Given the identification of flagella on BUA96 by transmission electron microscopy, we suggest that the whole genome sequence of this atypical HI strain be determined to confirm the presence of *fliC* and *flgH*, as well as to identify the other 40-50 genes required for production of flagella.

1. Carabarin-Lima A, Lozano-Zarain P, Castañeda-Lucio M, Martínez de la Peña CF, Martinez-Garcia J et al. Flagellar expression in clinical isolates of non-typeable *Haemophilus influenzae*. *J Med Microbiol* 2017;66:592-600.

2. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* 1995;269:496-512.

3. Negrete-Abascal E, Reyes ME, García RM, Vaca S, Girón JA et al. Flagella and motility in *Actinobacillus pleuropneumoniae*. *J Bacteriol* 2003;185:664–668.

4. Foote SJ, Bossé JT, Bouevitch AB, Langford PR, Young NM et al. The complete genome sequence of *Actinobacillus pleuropneumoniae* L20 (serotype 5b). *J Bacteriol* 2008;190:1495-6.