Type IV secretion genes in *cagE*-negative *Aggregatibacter actinomycetemcomitans* serotype b

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ABSTRACT

Aggregatibacter actinomycetemcomitans is a Gram-negative bacterium that plays an important role in the development of aggressive periodontitis. The *cagE* gene, which encodes a putative exotoxin, has been found to be present in highly leukotoxic serotype b strains. Both in the JP2 genotype, in which there is a 530-bp deletion in the leukotoxin operon's promoter region, and in the non-JP2 genotype, and it was recently shown that the *cagE* gene could serve as a genetic marker for highly leukotoxic serotype b strains. It was also noticed that the *cagE* gene and the *virB4* gene did not seem to be found in the same strain. The aim of this study was to determine whether the *cagE* negative strains of *A. actinomycetemcomitans* serotype B harboured the *virB4* gene and vice versa. We hypothesize that the *virB4* gene would be present in the serotype b samples that lacked the *cagE* gene and vice versa. In order to screen the samples for the presence of the *virB4* gene conventional polymerase chain reaction (PCR) and quantitative PCR (qPCR) was used. Indeed, we found that the *cagE* gene and the *virB4* gene seldom co-existed in the same strain of *A. actinomycetemcomitans*, i.e. it can function as a marker for non-*cagE* genotype strains. However, it could also be concluded that it was common for a strain to lack both of these genes.

INTRODUCTION

Periodontitis is a chronic, inflammatory disease that causes the loss of periodontal supporting connective tissue and surrounding alveolar bone and can, if left untreated subsequently lead to tooth loosening and eventually tooth loss (Pihlstrom et al, 2005). Periodontitis is the most common cause of tooth loss globally (Darveau, 2010) and is recognized in two principal forms: chronic and aggressive periodontitis (Papapanou et al, 2017).

The chronic variety is the most common and is estimated to affect a majority of the worldwide population aged over 35 (Moore et al, 1983) while the aggressive form of the disease affects less than 1 % of the worldwide population and is found almost exclusively in adolescents and young adults (Höglund et al, 2012). Periodontitis is a multifactorial disease, with risk factors including subgingival biofilm, genetic factors, lifestyle - such as smoking-, systemic diseases, and genetic predisposition. In adolescents, it is likely that the genetic make-up of an individual in combination with microorganisms plays the greatest role in the development of the disease (Loos et al, 2015). Periodontitis is a polymicrobial infectious disease, rarely ever associated

with the existence of a single or a few bacterial species. It is considered a microbial-shift disease and arises when the dental plaque community goes from being composed of mainly Gram-positive species to instead harbour mainly Gram-negative species (Berezow et al, 2011).

One of the most noticeable risk factors for developing aggressive periodontitis is the presence of *A. actinomycetemcomitans* (Höglund et al, 2012). *A. actinomycetemcomitans* is a small, facultative anaerobic, Gram-negative, non-motile bacterium. (Brigido JA et al, 2014). Currently there are seven identified serotypes of *A. actinomycetemcomitans*. (a-g). Serotype b has a particularly leukotoxic genotype, JP2, which is in many cases found in patients with African descent and which manifestation in adolescents strongly correlates with aggressive periodontitis (Höglund et al, 2013).

The leukotoxin of *A. actinomycetemcomitans* is a member of the so called RTX family (Repeats-in-toxin), a group of cytotoxins typically produced by various Gram-negative bacteria (Johansson, 2011).
As far as known, all strains of *A. actinomycetemcomitans* possess a complete leukotoxin operon, although some strains can produce much higher concentrations of the leukotoxin than others, for example JP2 (Henderson et al, 2010). It has been shown that there is a higher leukotoxicity in *A. actinomycetemcomitans* isolated from periodontally diseased subjects than in isolates derived from periodontally healthy subjects (Zambon et al, 1983). The leukotoxin is a pore forming toxin thought to, in low concentrations, form small pores in the affected cell membrane which triggers the cell's self-destruct apparatus consequently causing the cell to undergo apoptosis.
The target for the Leukotoxin has been identified as the lymphocyte function associated antigen 1 receptor (LFA-1 receptor) a heterodimer comprised of two subunits; an αL subunit and a β2 subunit. The LFA-1 receptor is present on the cell surfaces of leukocytes and is involved in the adherence to antigen presenting cells. In higher concentrations the leukotoxin is thought to induce cell lysis due to the formation of LtxA-LFA-1 complexes on the target cell membrane which causes an immediate breakdown of the affected cell membrane. The leukotoxin targets cells with hematopoietic origin and has been shown to have affected polymorphonuclear leukocytes, monocytes, macrophages, lymphocytes and erythrocytes (Johansson, 2011).

Polymorphonuclear leukocytes (PMN) can phagocytize and kill *A. actinomycetemcomitans* provided that the strain of bacteria is not highly leukotoxic in which case not only will

leukotoxin kill the PMNs but it will also cause the polymorphonuclear leukocytes to release vesicles filled with proteolytic enzymes and also induce an unregulated secretion of matrix metalloproteinase 8 (MMP-8), a collagen cleaving enzyme that, under normal circumstances plays an important role in tissue remodelling. This abnormal release of MMP8 will lead to uncontrolled tissue degradation and contribute to the periodontitis progression (Johansson, 2011). In macrophages the leukotoxin can in addition to causing cell lysis also induce the activation of caspase-1 (Johansson, 2011). Caspase -1 is a cysteine protease that functions by way of cleaving the pro-inflammatory cytokine Il-1β precursor into a bioactive form (Winkler et al, 2015). Il-1β induces increasing levels of the protein receptor activator of nuclear factor kappa-B ligand (RANKL) which by way of binding to its receptor - receptor activator of nuclear factor κ B (RANK) present on osteoclast progenitor cells causes the osteoclast progenitor cells to differentiate into active osteoclasts (bone resorbing cells). Thus Il-1β induces bone resorption (Sonnenschein et al, 2015).

The JP2 genotype is distinguished from other so called non-JP2 strains by a 530 base pair deletion in the promoter region of the ltx gene - the gene that encodes the leukotoxin, which may be the reason why JP2 produces such high concentrations of the leukotoxin. Subsequently the risk of periodontal attachment loss is increased in carriers of the JP2 clone (Haubek et al, 2008). However, there are highly leukotoxic strains that are non-JP2 genotype. In a recent study by Johansson et al. (2017) it was discovered that the *cagE* gene was present in highly leukotoxic serotype b strains of *A. actinomycetemcomitans*, both of the JP2 and non-JP2 variety, and it was proposed that the *cagE* gene could serve as a genetic marker for highly leukotoxic serotype b strains.

There exist at least six major classes of secretion systems in Gram negative bacteria, named Type I to Type VI secretion system (T1SS-T6SS). T4SS is almost always composed of at least 12 proteins: VirB1- VirB11 and VirD4 (Waksman et al, 2014) and take part in bacterial conjugation, the release and uptake of DNA, and in the injection and secretion of effector proteins and toxins into eukaryotic cells (Waksman et al, 2014). The genes encoding this conjugation machinery are termed Mating Pair Formation genes (MPF) and have been found in the plasmid pVT745 and is also frequently discovered in various isolates of *A. actinomycetemcomitans* (Liu et al, 2018). It was recently observed (Johansson et al, 2017) that the gene encoding the VirB4 protein (an ATP:ase homologue), in serotype b strains show strong homology to the *CagE* C-terminus, and that the gene encoding the VirB1 protein (lytic

transglycosylase homologue), exhibit strong homology to the CagE N-terminus. Another observation from that study was that the *cagE* gene and the *virB4* gene did not seem to co-exist in the same strain. It was therefore speculated that CagE in fact may represent a fusion product of virB1 and virB4 (Johansson et al, 2017). However, only one confirmed *cagE*-positive strain was assessed for *virB4* and *virB1* in that work, which is the reason why this present study was carried out.

The aim of this study was to examine whether there exists a correlation between the lack of the *cagE* gene and the presence of the T4SS specific genes in serotype B strains of *A. actinomycetemcomitans*. Can the *virB4* gene be used as a diagnostic marker to determine if the patient is a carrier of a low leukotoxic *A. actinomycetemcomitans* strain? We hypothesize that in the serotype b strains of *A. actinomycetemcomitans* where the *cagE* gene is present, the genes encoding the T4SS (*virB4*) are not present and vice versa i.e. these two genes do not co-exist.

MATERIALS AND METHODS

Bacterial strains and DNA extractions

For this study, DNA had been isolated previously from the collections of *A. actinomycetemcomitans* strains in the laboratory of Oral Microbiology. The DNA samples used were either boiled bacterial colonies in water or preparations done using a commercial kit. These samples came from *A. actinomycetemcomitans* serotype b strains (Table 1) and from a local, Umeå collection of strains (Table 1-4). The samples were used in PCR and qPCR as described below.

PCR analysis

PCR is a laboratory technique where an enzyme, Taq polymerase is used to, in cycles, amplify a specific part of the template DNA. In each cycle the target sections of the DNA are doubled which causes an exponential amplification of the **target** DNA segment. The primers used for the *virB1* gene were ltg_R (5´-GTTTTTAATCAATCTTCCTGATTG -3´) and *cagE*_F (Teng et al, 2003) (5´GGATCCGTCCCTGAAATTTTATTAGCTTG-3´). Primers used for the *virB4*

gene were atpF1 (5´-GTGCAGAAGCCTGTATTCGTGC-3´) and atpF2 (5´-CCAGTCATTAGTGGCTTCGCC-3´).
For the PCR analysis Ready-To-Go-PCR PCR beads (GE Healthcare, Amersham, Bucks, UK) was used and the mixture was as follows:

- 22 uL H2O
- 1 uL Forward Primer
- 1 uL Reverse Primer

Then 1 uL DNA sample was added.
The cycling conditions for the PCR analysis were as follows: 94°C for 1 min, thereafter 35 sets of 94°C for 30 s, 54°C for 30 s, 72°C for 1 min and then 72°C for 7 min. PCR products were then analysed in agarose gel electrophoresis.

qPCR analysis

Quantitative polymerase chain (qPCR) reaction, is a lab technique that monitors the amplification of the template DNA and differs from conventional PCR because it registers the amplification of the specific DNA segment in real time, which is why it is also referred to as Real-Time PCR, and not, as in conventional PCR at the end. When performing qPCR analyses the target DNA segments are dyed with a fluorescent dye that is registered during the amplification. The more amplicon DNA the more fluorescence is emitted.

In order to prepare the samples for PCR analysis the KAPA SYBR® FAST qPCR Kit (Kapa Biosystems, Wilmington, MA, USA) kit was used and the mixture was as follows:

- 5 uL SYBR Green
- 1 uL atpF
- 1 uL atpR
- 2 uL MQ

And then 1 uL DNA sample was added. The cycling conditions for qPCR were as follows:

1. (Hold) 95 degrees for 10 min,
2. (Cycling) 95 degrees for 10 seconds, 56 degrees for 5 seconds, 72 degrees for 22 seconds, repeated 45 times.

3. (Melt) Ramp from 50 degrees to 95 degrees, rising by 1 degree each step, wait for 90 seconds of pre-melt conditioning on first step. Wait for 15 seconds for each step afterwards.

The PCR primers for the *virB4* gene were atpF1 (5´-GTGCAGAAGCCTGTATTCGTGC-3´), and atpF2 (5´-CCAGTCATTAGTGGCTTCGCC-3´). After the samples had been put through the qPCR analysis they were analysed for the presence of the *virB4* gene using agarose gel electrophoresis.

Agarose gel electrophoresis

PCR and qPCR products were run on agarose gels. The *virB4 PCR product* about 900 base pairs (bp) and that of *virB1* gene approximately 630 bp. Their presence in the agarose gel was verified by positive control samples and/or a DNA ladder.

RESULTS

PCR screening of virB1 and *virB4* in serotype b strains

First, DNA preparations from 18 reference strains of *A. actinomycetemcomitans* serotype b were analysed by PCR, using primers specific for *virB1* and *virB4*, respectively, and products were then run on agarose gels (Figure 1). Their *cagE* genotype had been determined previously (Johansson et al, 2017). The 16 strains that were positive for the *cagE* gene were all negative for both the *virB4* and the *virB1* gene (Table 1). The two strains that were negative for the *cagE* gene were positive for both the *virB4* gene and the *virB1* gene. In conclusion all the 18 samples harboured either both the *virB4* and the *virB1* genes or lacked these genes and carried instead the *cagE* gene.

qPCR screening of *virB4* in serotype b strains

Next, to analyse the presence of *virB4* in a larger selection of strains, qPCR was used to screen DNA preparations from a local collection of *A. actinomycetemcomitans* isolates sampled at Oral Microbiology. In the main study a total of 102 samples were analysed regarding the presence of the *virB4* gene. Their *cagE* genotype had been determined previously in the laboratory (Johansson, Oscarsson et al., unpublished data). In the qPCR, melting analysis was performed to get an idea of the specificity of the PCR amplification. The qPCR melting points were

somewhat difficult to determine. However, the large majority, 19 of the 23 cases where a strain was determined to be *virB4*-positive, there was a melting top in the range of 80-82 degrees (Supplementary Table 1). Therefore, to confirm that the *virB4* qPCR product had the expected size, all reactions were run on agarose gels (Figure 2).

To summarize the qPCR results, 46 of the samples were positive for the *cagE* gene and negative for the *virB4* gene, whereas 21 samples were negative for the *cagE* gene while positive for the *virB4* gene. In 33 of the samples neither of the two genes could be detected, and in 2 of the samples both the *cagE* gene and the *virB4* gene were found to co-exist (Figure 3A). Thus, in a majority, 67% of the cases only one of the genes was found. However, in rare cases, in 2% of the samples the genes were found to co-exist whereas remaining samples were lacking both genes.

Thus, we have identified four genotypes based on *cagE*/*virB4* (*cagE+/virB4-*, *cagE-/virB4+*, *cagE-/virB4-*, *cagE+/virB4+*). The next step was to examine whether these groups of strains are homogenous, belonging to the same arbitrarily primed (AP)-PCR types.

In addition to *cagE*, all strains in the tested collection had been previously genotyped using AP-PCR according to a known procedure (Claesson et al, 2017). Of the *cagE*-positive/*virB4*-negative samples, the vast majority, 93% belonged to the AP-PCR type 1, 4% belonged to the AP-PCR type 5 and 2% was of the AP-PCR type 4 (Figure 3B and Supplementary Table 2).

Of the *cagE*-negative/*virB4*-positive samples 48% belonged to the AP-PCR type 2, 19% were of the AP-PCR type 6 variety, 14% of the AP-PCR type 3, and the other samples were evenly distributed, 5% each between AP-PCR type 1, AP-PCR type 4, AP-PCR type 5, and AP-PCR type 7 (Supplementary Table 3 and Figure 3C). Of the *cagE* negative/*virB4* negative 36 percent of the samples belonged to the AP-PCR type 2, 21% was of the AP-PCR type 4, 12% of the AP-PCR type 5, 9% were of AP-PCR type 6, 9% were of AP-PCR type 7, 9% of the AP-PCR type 3 and 3% were of the AP-PCR type 1 (Supplementary Table 4 and Figure 3D). Finally, of the two *cagE*-positive/*virB4*-positive samples both were of AP-PCR type 1 (Supplementary Table 5 and Figure 3E). Thus, in conclusion, *cagE*-positive isolates appear to represent a rather homogeneous group of strains where almost all are *virB4*-negative and belong to AP-PCR type 1. In contrast, *cagE*-negative strains appear to be a more diverse group, consisting of both *virB4* -positive and *virB4* -negative strains. In both these groups of *cagE*-negative strains, however, AP-PCR type 2 is the most common.

DISCUSSION

The focal point of this study was to evaluate whether the T4SS-associated genes *virB4* and *virB1* might be useful as genetic markers for *A. actinomycetemcomitans* serotype b strains, exhibiting low leukotoxicity. The hypothesis that highly leukotoxic and virulent strains, belonging to the *cagE* genotype, would be *virB4* - and *virB1*-negative was based on recent observations in this laboratory, in which it was speculated that the *virB1* and *virB4* genes originated from the *cagE* gene and this would explain why the two genes rarely co-exist (Johansson et al, 2017). However, as only one *cagE*-positive strain was analysed for *virB4* and *virB1* in that study, we wanted to use a larger collection of strains, representing both the *cagE*-positive and *cagE*-negative genotype. We therefore studied a locally sampled collection of 102 serotype b strains both negative and positive for the *cagE* gene to determine whether the *cagE* negative strains of *A. actinomycetemcomitans* serotype b harboured the *virB4* gene. We hypothesized that the *virB4* gene would be present in only the serotype b samples that lacked the *cagE* gene and vice versa. This was true for a majority of the samples tested, 68 of 102, in which only one of the two genes *cagE* and *virB4* could be found. In the rest of the samples the two genes were either both absent or in very few cases they were found to co-exist. Therefore, the hypothesis that the two genes cannot co-exist is mostly true.

The two cases where the *cagE* gene and the *virB4* gene are both present can perhaps be explained if one of the two genes is found on a plasmid. Indeed, T4SS genes such as *virB1* and *virB4* have been found to be encoded on plasmids in some strains, rather than on the chromosome (Novak et al, 2001). Thus, co-existence is evident in the JP2 genotype strain HK1651, which carries both *virB1* and *virB4* on a plasmid (Liu et al, 2018) and *cagE* on the chromosome (Johansson et al, 2017). Interestingly, none of the JP2 genotype reference strains tested in this study was scored as *virB4* -positive, despite that the DNA sequence of the plasmid-encoded *cagE* of HK1651 to 100% matches the sequences of the *cagE* PCR primers used. This suggests that the plasmid might have been lost in the strains tested. Samples where both the genes were absent might be explained similarly, i.e. a *cagE*-negative strain losing its plasmid encoding *virB4*. Assuming that in some *cagE*-negative strains, the *virB4* gene was in fact originally on a plasmid, it could have been lost during cultivation of *A. actinomycetemcomitans*, which has been proven to have the ability to during *in vitro* cultivation lose a plasmid that is not needed (Sreenivasan et al, 1994). We believe that the DNA preparation methods used for

the samples analysed (QiaGen genomic DNA kit or boiled bacterial colonies) would not themselves result in samples lacking plasmid DNA. As far as known, there is no evidence yet of *cagE* being encoded on a plasmid in this species. It is also possible that *cagE*-negative strains exist, having sequence alterations where the PCR primers are meant to bind in *virB4* and/or *virB1*. This remains to be tested using additional sets of PCR primers, and/or whole genome sequencing. However, no such strain has yet been identified by analysing the *A. actinomycetemcomitans* genomes available in databases (Johansson et al, 2017).
The qPCR assay could also be optimized to ensure that samples do not need to be put through agarose gel electrophoresis in order to determine the *virB4* genotype.

In conclusion, these test results have revealed that in a majority of serotype b strains the *cagE* and the *virB4* gene normally do not co-exist. As *cagE* is strongly linked to highly leukotoxic strains (Johansson et al, 2017), this could mean that the presence of the *virB4* gene in a strain of *A. actinomycetemcomitans* serotype b could indicate that the patient is a carrier of a low-leukotoxic strain. To be able to distinguish between high and low leukotoxic strains could help ensure that carriers are early on provided with proper periodontal care.

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APPENDIX

TABLES

Strain	*cagE*	*virB4*	*virB1*	JP2
J1	+	-	-	+
J5	+	-	-	+
J6	+	-	-	+
J8	+	-	-	+
J15	+	-	-	+
J22	+	-	-	+
J27	+	-	-	+
J30	+	-	-	+
J34	+	-	-	+
J38	+	-	-	+
HK908	-	+	+	-
HK912	+	-	-	-
575G	+	-	-	-
443G	+	-	-	-
605G	+	-	-	-
638G	+	-	-	-
486G	+	-	-	-
AHN9351	-	+	+	-

Table 1.

Table listing the 18 strains analysed by PCR in the pilot study. All 16 of the strains that were positive (+) for the *cagE* gene were negative (-) for both the *virB4* and *virB1* genes. In contrast, the two strains that were negative for the *cagE* gene were positive for both the *virB4* and *virB1* genes. Also, JP2 (+) or non-JP2 (-) genotype is indicated.

FIGURES

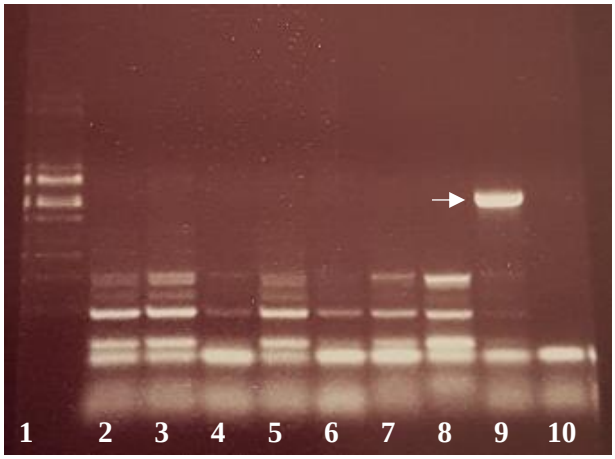

Figure 1. PCR analysis of *virB4* in *A. actinomycetemcomitans* serotype b strains.
Well nr 1 contains the DNA ladder. The *cagE*-positive samples were loaded as follows: 2: J1, 3: J5, 4: J8, 5: J15, 6:J22, 7:J27, 8: 575G. Well nr 9 contains a *cagE*-negative sample (strain ANH9381) and well nr 10 contains a sample where H_2O acted as a negative control in the PCR. The band corresponding to the *virB4* gene product (900 bp) can be seen in lane 9, and is indicated with an arrow.

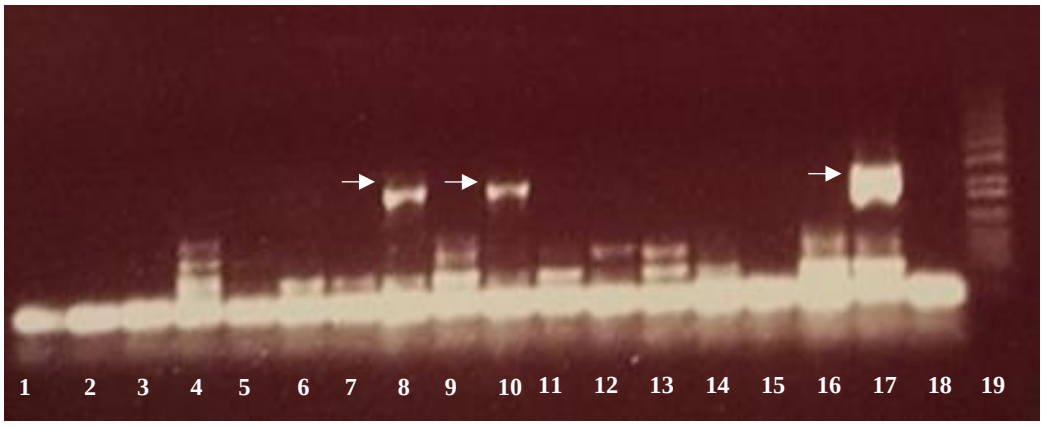

Figure 2. qPCR analysis of *virB4* in *A. actinomycetemcomitans* serotype b strains
PCR banding pattern of various *A. actinomycetemcomitans* serotype b strains from the local collection after qPCR analysis of *virB4*. Lane nr 17 harbours the positive control sample (strain ANH9381), lane nr 18 contains H_2O acting as a negative control sample and well nr 19 contains

the DNA ladder. Arrows show the banding indicating the presence of the *virB4* gene in the samples loaded in lane 9, and 11.

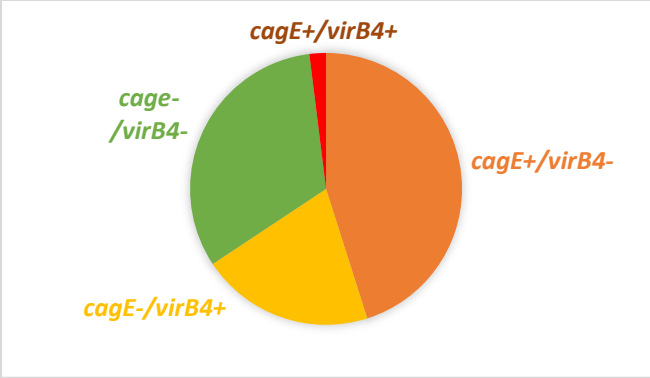

Figure 3A

Circle diagram showing the prevalence of the four different *cagE/virB4* genotypes among the 102 analysed *A. actinomycetemcomitans* serotype b strains. A majority of the strains were positive for only one of the genes

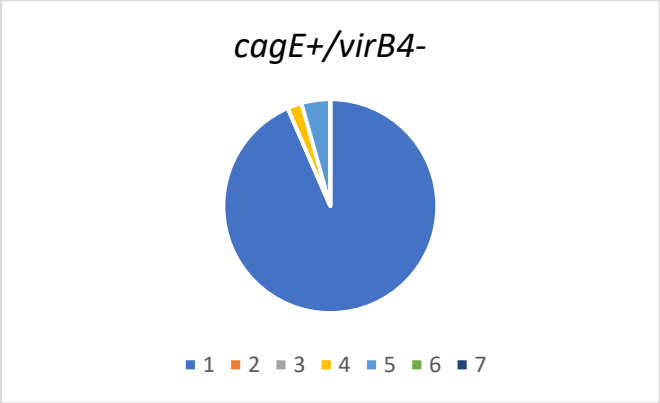

Figure 3B

Circle diagram showing how great a proportion of the *cagE* positive (+) and *virB4* negative (-) samples that belong to the different AP-PCR types. Blue: AP-PCR type 1. Purple : AP-PCR type 4. Red: AP-PCR type 5. A large majority of the strains were AP-PCR type 1.

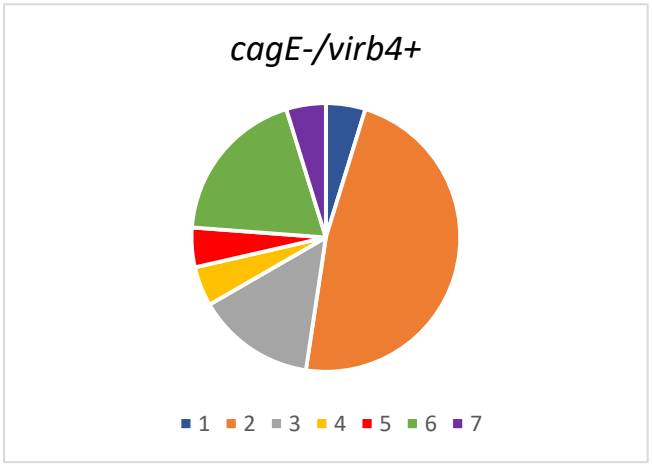

Figure 3C

Circle diagram showing how great a proportion of the *cagE* negative (-) and *virB4* positive (+) samples that belong to the different AP-PCR types. Blue: AP-PCR type 1. Orange: AP-PCR type 2. Grey: AP-PCR type 3. Yellow: AP-PCR type 4. Red: AP-PCR type 5. Green: AP-PCR type 6. Purple: AP-PCR type 7. AP-PCR type 2 was the most common in this heterogeneous genotype.

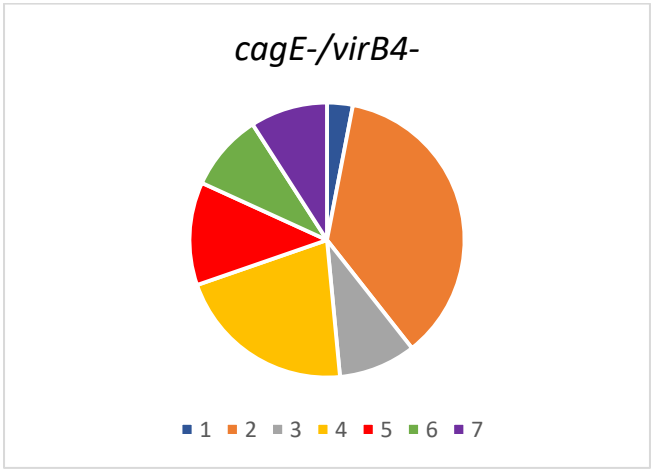

Figure 3D

Circle diagram showing how great a proportion of the *cagE* negative (-) and *virB4* negative (-) samples that belong to the different AP-PCR types. Blue: AP-PCR type 1. Orange: AP-PCR type 2. Grey: AP-PCR type 3. Yellow: AP-PCR type 4. Red: AP-PCR type 5. Green: AP-PCR type 6. Purple: AP-PCR type 7. AP-PCR type 2 was the most common in this heterogeneous genotype.

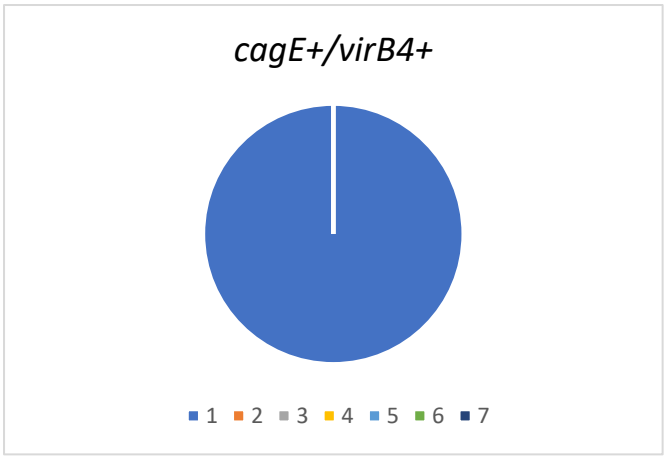

Figure 3E

Circle diagram showing how great a proportion of the *cagE* positive (+) and *virB4* positive (+) samples that belong to the different AP-PCR types. The two strains with this genotype were both AP-PCR type 1 (blue).

SUPPLEMENTAL APPENDIX

≤ 80 °C	>80-81 °C	>81-82 °C	>82 °C
3	7	12	1

Supplementary Table 1.

Table showing the melting temperatures of the *virB4* qPCR products in the 23 samples determined to be *virB4*-positive.

cagE+/virB4-	
strain	AP-PCR
11	1
89	5
134	1
136	1
138	1
139	1
143	1
144	1
146	1
148	4
149	1
152	1
167	1
172	1
184	1
186	1
194	1
195	5
204	1
209	1
212	1
214	1
221	1
244	1
248	1
251	1
262	1
281	1
296	1
317	1
318	1
321	1
327	1
335	1
348	1
352	1
354	1
358	1
360	1
364	1
374	1
393	1
407	1
408	1
409	1
411	1

Supplementary Table 2

Table showing the strains that were identified as positive (+) for the *cagE* gene and negative (-) for the *virB4* gene along with each strain's corresponding AP-PCR genotype.

cagE-/virB4 +	
strain	AP-PCR
14	2
27	7
35	6
54	5
73	6
78	2
107	3
137	2
173	3
185	2
234	2
255	2
261	6
311	2
320	2
356	3
400	4
405	6
410	2
413	2
415	1

Supplementary Table 3

Table showing strains identified as negative (-) for the *cagE* gene and positive (+) *virB4* gene along with the strain's corresponding AP-PCR genotype.

cagE-/virB4 -	
Strain	AP-PCR
12	5
62	1
66	5
67	7
80	2
99	4
108	7
112	3
128	6
141	4
145	2
161	2
177	2
179	3
189	4
196	2
200	4
201	2
208	4
219	7
260	5
266	6
275	2
279	4
280	5
282	2
290	2
310	2
314	2
329	2
346	6
391	3
403	4

Supplementary Table 4

Table showing the strains that were identified as negative for the both *cagE* gene and the *virB4* gene along with each strain's corresponding AP-PCR genotype.

cagE+/virB4 +	
Strain	AP-PCR
135	1
147	1

Supplementary Table 5

Table showing the strains that were identified as positive for both the *cagE* gene and the *virB4* gene along with each strain's corresponding AP-PCR genotype.