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# Isolation and comparison of *Arcanobacterium hippocoleae* isolates from the genital tract of 15 mares

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ABSTRACT

The present study reports the isolation of *A. hippocoleae* from genital swabs of 15 apparently healthy mares (at least one had an abortion one month earlier) and describes the genotypic and phenotypic characterisation of these strains. The mares were of eight different breeds with a thoroughbred dominance and came from 11 breeding farms located in the French region of Brittany. 16S rRNA gene sequencing confirmed the species' identification by comparing it with reference strain *A. hippocoleae* CIP 106850$^T$. Some degree of natural divergence within *A. hippocoleae* was observed by 16S rRNA sequencing (two 1,002-pb sequences), MALDI-TOF MS typing (two groups), a CAMP test (three different intensities of haemolysis from CAMP-positive results) and API® Coryne system (five profiles). The strains were all susceptible to the antimicrobials tested. A national prevalence survey would be required to estimate the frequency of *A. hippocoleae* carriage in mares and stallions and to verify the presence of *A. hippocoleae* outside the French region of Brittany, which is the only one found to be affected in the current study, probably because the isolates were recovered from a single field laboratory in this region.

## 1. Introduction

*Arcanobacterium hippocoleae*, a facultative anaerobic, non-acid-fast, Gram-positive irregular-shaped rod bacterium, was first described in 2002 from a mare's vaginal discharge and was assigned to the genus *Arcanobacterium* (Collins et al., 1982; Yassin et al., 2011) based on phylogenetic and phenotypic characterisation (Hoyles et al., 2002). According to this first reported case, the pathological significance of *A. hippocoleae* was unclear since strain M401624/00/2$^T$ (= CIP 106850$^T$ = DSM 15539$^T$ = CCUG 44697$^T$) was isolated in a mixed culture with a *Corynebacterium* sp. and coagulase-negative staphylococci. Thereafter, three other isolations of *A. hippocoleae* were reported: (i) in 2003, in a pure culture from horse urine without any descriptive information about the horse, clinical significance and isolate (Cai et al., 2003); (ii) in 2008, in numerous colonies observed by histological examination following a case of placentitis and stillbirth in a mare (Bemis et al., 2008); and (iii) in 2017, from the uterus swab of an apparently healthy mare (Wickhorst et al., 2017). Thus, the isolation of *A. hippocoleae* appears to be rare and seems to be host-adapted to the reproductive tract of horses. The role this species plays as commensal bacterium, whilst occasionally acting as an opportunistic pathogen in horses, remains unclear.

During the 2017 horse breeding season, the frequent detection of unusual strains isolated through official CEM tests was reported by LABOCEA35 to the national reference laboratory for contagious equine metritis (CEM), the ANSES Dozulé laboratory for equine diseases; these strains had been classified as *A. hippocoleae* with a high confidence level according to matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) analysis during routine microbiological diagnosis. The aim of the present study is to report on the isolation of *A. hippocoleae* from the genital swabs of 15 apparently healthy mares (at least one mare had an abortion one month earlier) in the French region of Brittany. Alongside identification and characterisation of the 15 *A. hippocoleae* strains compared to reference strain CIP 106850$^T$, we investigated intra-species variability through 16S rRNA gene sequencing and phenotypic methods including MALDI-TOF MS typing, the Christie-Atkins-Munch-Peterson (CAMP) test with *Staphylococcus aureus* and the API® Coryne system.

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**Table 1**
Origin of the 15 *A. hippocoleae* strains investigated in the present study.

| Summary of mares | | | | | Summary of *A. hippocoleae* strains | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Mare ID | Birth year | Breed | Breeding farm ID[a] | Date of sampling (day.mo.yr) | **Strain ID**[b] | | Sample sites[c] | Colony counts | Type of culture |
| 1 | 1996 | Thoroughbred | 1 | 13.02.2017 | **1** | (17/1260 = MBE 713) | CS | > 100 | Pure culture |
| 2 | 2011 | Thoroughbred | 2 | 16.02.2017 | **2** | (17/1409 = MBE 714) | CS | > 100 | Mixed culture |
| 3 | 2006 | Thoroughbred | 3 | 07.03.2017 | **3** | (17/2000 = MBE 715) | CFS | > 100 | Mixed culture |
| 4 | 2006 | Anglo-Connemara | 4 | 09.03.2017 | **4** | (17/2056 = MBE 716) | CS, C | 1-10 | Majority culture |
| 5 | 2014 | Thoroughbred | 5 | 09.03.2017 | **5** | (17/2065.01 = MBE 717) | CS, C | > 100 | Majority culture |
| 6 | 2003 | Thoroughbred | 5 | 09.03.2017 | **6** | (17/2065.02 = MBE 718) | CS | 10-100 | Mixed culture |
| 7 | 2007 | Thoroughbred | 6 | 20.03.2017 | **7** | (17/2351 = MBE 719) | CS, C | > 100 | Mixed culture |
| 8 | 2002 | Friesian | 7 | 22.03.2017 | **8** | (17/2419.01 = MBE 720) | CS | > 100 | Majority culture |
| 9 | 2005 | Pony | 7 | 22.03.2017 | **9** | (17/2419.02 = MBE 721) | CS | > 100 | Majority culture |
| 10 | 2001 | Thoroughbred | 5 | 23.03.2017 | **10** | (17/2462 = MBE 722) | CS, C | > 100 | Pure culture |
| 11 | 2012 | Thoroughbred | 8 | 07.04.2017 | **11** | (17/2900 = MBE 725) | CS, C | > 100 | Pure culture |
| 12 | 2008 | Quarter Horse | 9 | 06.05.2017 | **12** | (17/3515 = MBE 735) | CS, C | > 100 | Majority culture |
| 13[d] | 2006 | Gypsy Cob | 10 | 11.05.2017 | **13** | (17/3576 = MBE 736) | CS, C | > 100 | Majority culture |
| 14 | 2000 | French Trotter | 11 | 26.06.2017 | **14** | (17/4580 = MBE 737) | CFS | > 100 | Majority culture |
| 15 | 2015 | Irish Cob | 10 | 14.09.2017 | **15** | (17/6203 = MBE 743) | CS, C | > 100 | Majority culture |

[a] All breeding farms are located in the French region of Brittany which covers four departments. Farm ID 6 is in the "Côtes d'Armor" department while all the others are in the "Ille et Vilaine" department.
[b] Strain identification from LABOCEA35 and ANSES bacterial collections are in brackets: (LABOCEA35 strain ID = ANSES strain ID).
[c] Sampling sites include the clitoral sinus (CS), clitoral fossa and sinus (CFS) and cervix (C). With the exception of the cervical sample from mare ID 7, which was *A. hippocoleae*-negative after seven days of incubation, all the sampling sites tested were *A. hippocoleae*-positive after three days of incubation at 37 ± 2 °C with 7% $CO_2$ in air. In presence of two positive sampling sites per animal, only the strain isolated from the cervix was conserved to be investigate in the present study.
[d] Abortion on 7 April 2017.

## 2. Materials and methods

### 2.1. Bacterial strains

The strains investigated in the present study included 15 *A. hippocoleae* (ID 1 to 15; Table 1) isolated in 2017 from the genital swabs of 15 apparently healthy mares during official CEM tests, plus reference strain *A. hippocoleae* CIP 106850$^T$, which was isolated from the first reported case of *A. hippocoleae* (Hoyles et al., 2002). It should be noted that mare ID 13 had an abortion one month earlier. CEM analyses were performed by LABOCEA35 (Fougères, France) according to French AFNOR standard NF U47–108 (AFNOR, 2012) which complies with the World Organisation for Animal Health (OIE, 2012). The classification as *A. hippocoleae* was based on MALDI-TOF MS analysis during routine bacteriological diagnosis.

The strains were maintained using CryoBeads™ (bioMérieux) at a temperature ≤ −65 °C. They were cultivated on ready-to-use 5% sheep blood agar (bioMérieux) incubated at 37 ± 2 °C in 7% $CO_2$ in air for 48 h in order to obtain colonies for phenotypic identification and 16S rRNA gene sequencing.

### 2.2. Phenotypic identification

An ID colour catalase test (bioMérieux) and the API® Coryne system (bioMérieux) were performed according to the manufacturer's instructions. In addition, haemolytic properties and CAMP reactions on 5% sheep blood agar were determined after 48 h of incubation at 37 ± 2 °C in 7% $CO_2$ in air for haemolytic properties and 5% $CO_2$ in air for CAMP reactions respectively. For the CAMP reactions, the *S. aureus* reference culture (from the LABOCEA35 collection of identified and characterised field strains) was used to visualise the effects of co-haemolysis; a distinct arrowhead of haemolysis at the intersection of the *S. aureus* colony streak and the *A. hippocoleae* colony streak was considered a positive result.

Minimum inhibitory concentrations (MIC) of various antimicrobial agents, including penicillin, vancomycin, gentamicin, erythromycin, doxycycline, tetracycline, clindamycin, trimethoprim/sulfamethoxazole and rifampicin, were determined with the broth microdilution method, according to the VET06 and VET08 standards of the Clinical and Laboratory Standards Institute (CLSI) for *Corynebacterium* spp. and Coryneforms (CLSI, 2017, 2018). Quality of the susceptibility testing was controlled using two American Type Culture Collection (ATCC) strains (*Escherichia coli* ATCC 25922 (for gentamicin) and *Streptococcus pneumoniae* ATCC 49619). MIC values, defined as the lowest antibiotic concentration at which 50% ($MIC_{50}$) and 90% ($MIC_{90}$) of each strain was inhibited, were determined using 96-well microtiter plates containing dehydrated antibiotic (TREK Diagnostic Systems Ltd) after 48 h of incubation at 37 ± 2 °C in 5% $CO_2$ in air and calculated for each *A. hippocolae* strain against each antibiotic.

MALDI-TOF MS analysis was performed on a Vitek MS™ instrument (bioMérieux) equipped with both IVD (In Vitro Diagnostic) and RUO SARAMIS™ (for Research Use Only) databases (bioMérieux). For the IVD analysis, spectra were obtained using the Vitek MS™ automation control and Myla software (bioMérieux) with the manufacturer's suggested settings. For each acquisition group, a standard (*Escherichia coli* ATCC 8739) was included to calibrate the instrument and validate the run according to the manufacturer's instructions. The spectra were analysed by the Vitek MS™ v3.1 IVD database that contains spectral profiles (closed database). The software compares the spectrum obtained to the expected spectrum of each organism or organism group (e.g. bacteria or fungi) and high confidence level ID was considered when the single species' probability of ID ≥ 60%. For the RUO analysis, spectra were generated using the Launchpad v2.8 software (bioMérieux) and compared to the SARAMIS™ v.4.14 database (open database). For Superspectra and dendrogram construction, the spectra of all the strains were imported into the SARAMIS Premium™ software package. Superspectra were then calculated using the SARAMIS™ SuperSpectrum tool (bioMérieux) according to the manufacturer's instructions, and the specificity of the potential biomarker masses was determined by comparison against the whole SARAMIS™ spectral archive (bioMérieux). A dendrogram was created based on whole spectra, with a single-link clustering algorithm and a binary mass list.

### 2.3. S rRNA gene sequencing

According to the existing 16S rRNA gene sequence of *A. hippocoleae*

CIP 106850$^T$ (Hoyles et al., 2002; GenBank accession number AJ300767), a fragment of 16S rRNA gene with an approximate size of 1350 bp was amplified from strain CIP 106850$^T$ as a control as well as from strains from ID 1 to 15 using oligonucleotide primers 5′-GAG TTT GAT CCT GGC TCA G-3′ and 5′-AGG CCC GGG AAC GTA TTC AC-3′. PCR amplifications were performed with a 2 µl DNA template previously extracted using the NucleoSpin® Tissue kit (Macherey-Nagel) and added to the following reaction mixture (48 µl): 10 pM [each] forward and reverse primers, 25 µl PCR Phusion High-Fidelity PCR Master Mix containing 1.5 mM $MgCl_2$, 200 mM [each] deoxynucleotide triphosphates and 1.25 U Thermoprime Taq DNA polymerase (Thermo Scientific, France) and PCR-grade water. Amplification conditions were as follows: an initial step of 3 min at 98 °C; 35 cycles, each consisting of 1 min at 94 °C, 45 s at 60 °C, and 1 min at 72 °C; then one final step of 5 min at 72 °C using a MasterCycler Flexid Nexus Gradient thermal cycler (Eppendorf). PCR products were purified and sequenced by GENEWIZ® (United Kingdom). Sequences were aligned using MEGA7 software (Tamura et al., 2013) and trimmed to the size of the shortest sequence to define nucleotide sequence similarities.

## 3. Results

*A. hippocoleae* strains were isolated in 2017 from the genital tract of 15 mares (Table 1). The mares came from 11 breeding farms located in two departments ("Côtes d'Armor" and "Ille et Vilaine") in the French region of Brittany, and sampled from February to September 2017 by eight different veterinarians (Table 1). These mares were apparently healthy, but to our knowledge, at least one (mare ID 13) had an abortion one month earlier. They were between 2 and 21 years old (median and standard deviation of 10.9 ± 5.1 years) and were from eight different breeds with a thoroughbred dominance (53%). One or two swab specimens were analysed per mare: one from the clitoral sinus with or without the clitoral fossa (n = 15), and the second from the cervix (n = 8). Apart from one cervical swab (mare ID 7), all the others were *A. hippocoleae*-positive after three days of incubation at 37 °C in 7% $CO_2$ in air; *A. hippocoleae* strains were mostly in numerous colonies in association with other microorganisms or in a pure culture (Table 1).

Invariably, all *A. hippocoleae* strains consisted of Gram-positive, irregular-shaped and non-branching rods, and the catalase reaction was negative. After transplanting onto 5% sheep blood agar and following two days of incubation at 37 °C in 7% $CO_2$ in air, the colonies were circular, entire, convex, shiny and grey, and a relatively narrow zone of haemolysis was observed; at the end of five days of incubation, the colonies appeared white and more extensive haemolysis could be observed. CAMP-positive reactions with *S. aureus* were always observed but *A. hippocoleae* CIP 106850$^T$ and strains ID 1, 2, 3, 4, 7, 11, 13, 14, 15 showed extensive haemolysis, whereas strains ID 5, 6, 9, 10, 12 had a narrow haemolysis zone, strain ID 8 being between the other two observations (Fig. S1). The results of the API® Coryne system showed five different profiles (Table S1) including one dominant profile, composed of *A. hippocoleae* CIP 106850$^T$ and strains ID 1, 2, 3, 4, 7, 11. The four other profiles varied by one to three characters versus the dominant profile, for a total of four out of the 19 characters tested: α-glucosidase reaction, β-glucosidase reaction, ribose fermentation and glycogen fermentation. Thus, they were "α-glucosidase reaction" negative (strains ID 5, 6, 8, 10 and 12), "β-glucosidase reaction" positive (strains ID 13–15), "ribose fermentation" positive (strains ID 5, 6, 8, 10 and 12) and variably "glycogen fermentation" negative or positive on two repeat testing (strains ID 6, 8, 9 and 10). The 15 *A. hippocoleae* of the present study and *A. hippocoleae* CIP 106850$^T$ were all susceptible to the antimicrobials tested (Table S2).

The MALDI-TOF MS analysis enabled identification to species level with significant score values > 75% using the Vitkek MS™ RUO database. The dendrogram generated by the SARAMIS™ software (Fig. 1) showed the presence of two groups of strains with some spectral variability within each group, respectively composed of strains ID 1, 2, 3, 4, 7, 8, 11, 13, 14, 15 and *A. hippocoleae* CIP 106850$^T$ (group A), and strains ID 5, 6, 9, 10 and 12 (group B).

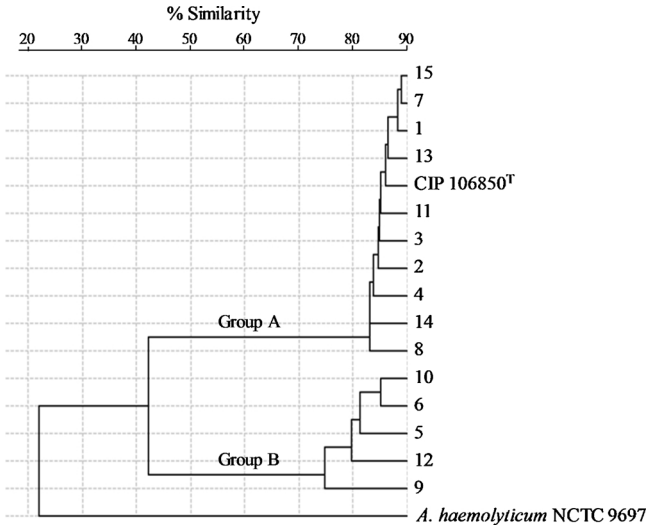


**Fig. 1.** MALDI-TOF MS dendrogram of 15 *A. hippocoleae* strains investigated in the present study and *A. hippocoleae* CIP 106850$^T$. *Arcanobacterium haemolyticum* NCTC 9697 was used as an outgroup. The dendrogram was generated by the SARAMIS™ software. Distance is displayed in % similarity along the *x* axis. Two MALDI-TOF groups were observed: (A) ID 1, 2, 3, 4, 7, 8, 11, 13, 14, 15 and CIP 106850$^T$; (B) ID 5, 6, 9, 10, 12.

16S rRNA gene sequencing confirmed identification to species level and showed the presence of two groups of strains characterized by two different 1,301-bp 16S rDNA sequences, named sequences A and B, with 99% similarity between them. Both groups of strains are respectively composed of strains ID 1, 2, 3, 4, 7, 8, 11, 13, 14, 15 and the reference *A. hippocoleae* CIP 106850$^T$ (sequence A), and strains ID 5, 6, 9, 10 and 12 (sequence B). The 16S rRNA gene sequences of strains ID 1–15 were submitted to GenBank and registered under accession numbers MH796227 to MH796241. These sequences were used to generate a phylogenetic tree showed Fig. 2.

Fig. 3 shows the five different patterns observed in the distribution

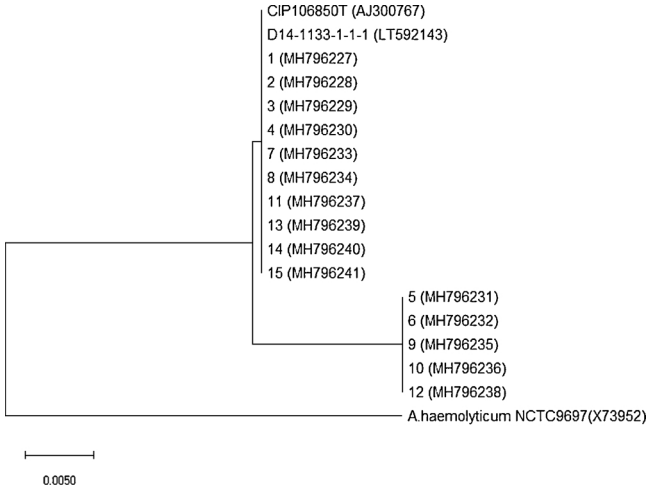


**Fig. 2.** Dendrogram of the 16S rRNA genes of 15 *A. hippocoleae* strains investigated in the present study and *A. hippocoleae* CIP 106850$^T$. The dendrogram was generated with cluster tree neighbour-joining analysis using the MEGA software version X (http://www.megasoftware). *A. haemolyticum* NCTC 9697 was used as an outgroup. Scale bar indicates 0.05 substitutions per 100 base positions. Numbers at tree nodes are bootstrap values from 1000 repetitions. The accession number of the 16S rDNA sequence is in brackets behind the ID of strains.

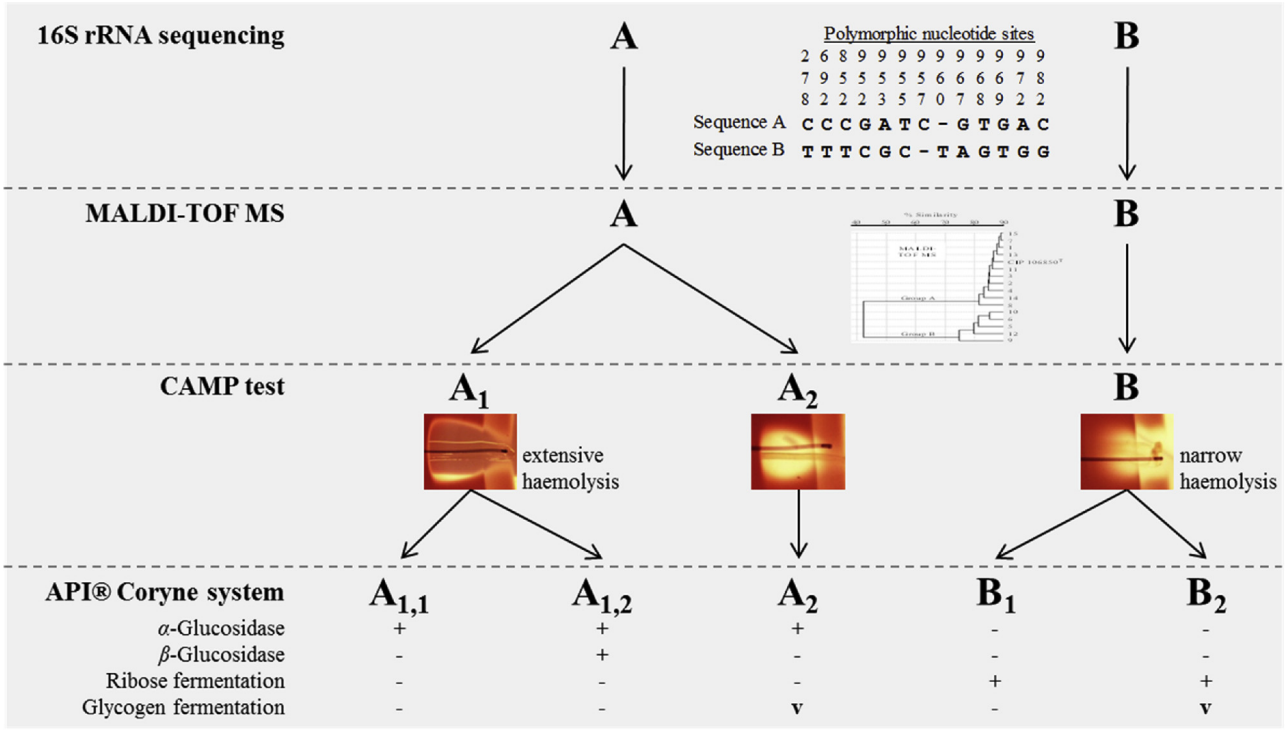


**Fig. 3.** Schematic distribution of *A. hippocoleae* strains and associated horse breeding farms according to the compilation of 16S rRNA gene sequences, MALDI-TOF groups, CAMP-positive reactions and API® Coryne profiles.

of the *A. hippocoleae* strains and associated horse breeding farms according to the compilation of phenotypic and 16S rRNA gene sequencing results. Dominant pattern $A_{1,1}$, composed of strains ID 1, 2, 3, 4, 7, 11 and *A. hippocoleae* CIP 106850$^T$, differs only from pattern $A_{1,2}$ (strains ID 13, 14, 15) by the β-glucosidase reaction and from pattern $A_2$ (strain ID 8) by the intensity of haemolysis, the CAMP-positive reaction and glycogen fermentation. On the other hand, dominant pattern $A_{1,1}$ is very different from pattern $B_2$ (strains ID 6, 9, 10) which, in turn, differs only from pattern $B_1$ (strains ID 5, 12) by glycogen fermentation. The farms ID 5, 7 and 10 contained more than one animal infected with *A. hippocoleae* (Table 1); in this case, we observed either one (farm ID 10) or two (farms ID 5 and 7) patterns of *A. hippocoleae* strains isolated from multiple animals within a same farm.

## 4. Discussion

The present study reports the isolation of *A. hippocoleae* from the genital tract of 15 apparently healthy mares. This bacterium was isolated in the 2017 horse-breeding season, during official CEM tests on 11 different farms located in two departments of the French region of Brittany. Eight different breeds, with thoroughbred dominance, were involved. The significant isolates, recovered in pure or mixed cultures from 22 out of 23 genital swabs, were identified as *A. hippocoleae* by MALDI-TOF MS and additionally identified by sequencing the 16S rRNA gene. To our knowledge, the presence of *A. hippocoleae* has previously been reported only four time in the literature (Bemis et al., 2008; Cai et al., 2003; Hoyles et al., 2002; Wickhorst et al., 2017), the pathological significance of *A. hippocoleae* being assigned to a case of placentitis and stillbirth in a mare (Bemis et al., 2008) and possibly causing a case of vaginal discharge in a mare (Hoyles et al., 2002).

Some degree of natural divergence within *A. hippocoleae* was observed by 16S rRNA sequencing and phenotypic methods including MALDI-TOF MS typing, the CAMP test and the API® Coryne system. The alignment of 16S rDNA gene sequences and the comparison of MALDI-TOF MS spectral profiles were consistent and categorised the strains into two groups, arbitrarily named A (strains ID 1, 2, 3, 4, 7, 8, 11, 13, 14, 15 and *A. hippocoleae* CIP 106850$^T$) and B (strains ID 5, 6, 9, 10 and 12); groups A and B further diverged on α-glucosidase reaction and ribose fermentation. Diversity was also observed within both groups. Indeed, the degree of haemolysis observed through CAMP-positive reactions was mostly consistent with 16S rRNA gene sequencing and MALDI-TOF MS categorisation, but strain ID 8 showed less extensive haemolysis than the others in group A. Furthermore, variable results were obtained for β-glucosidase reaction and glycogen fermentation, finally resulting in three different patterns within group A and two different patterns within group B. It should be noted that the categorisation within groups A and B could be further debated since degree of haemolysis observed through CAMP-positive reactions and variable results on repeat testing of glycogen fermentation probably do not provide a strong basis to allow this categorisation. Nevertheless, the quality of our results was validated through comparison with the 16S rDNA sequence and phenotypic characters previously reported for *A. hippocoleae* CIP 106850$^T$ (Hijazin et al., 2013; Hoyles et al., 2002). The observation of variable results on repeat testing has also been reported in the literature for the β-galactosidase reaction, the *N*-acetyl-β-glucosaminidase reaction and ribose fermentation from *A. hippocoleae* D14-1133-1-1-1 (Wickhorst et al., 2017).

Previous data from literature already indicated some degree of

natural divergence in the 16S rRNA gene (98% similarity over a 1,002-pb sequence), α-glucosidase, β-galactosidase and *N*-acetyl-β-glucosaminidase reactions, and ribose fermentation between *A. hippocoleae* CIP 106850$^T$ (Hoyles et al., 2002) and the *A. hippocoleae* strain reported by Bemis et al. (2008). Likewise, *A. hippocoleae* CIP 106850$^T$ and D14-1133-1-1-1 (Wickhorst et al., 2017) differed on five phenotypic characters which were weak positives for strain D14-1133-1-1-1 but positive (n = 2) or negative (n = 3) for strain CIP 106850$^T$; nevertheless both strains remained very similar according to MALDI-TOF MS results, other phenotypic characters reported and 99.6 to 100.0% similarities in five molecular targets including the 16 S–23 S rRNA intergenic spacer region and the 16S rRNA gene. Taking into account the literature and the present API® Coryne results, the diversity of the *A. hippocoleae* species is based on β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase and β-glucosidase reactions, and ribose, maltose, sucrose and glycogen fermentations. On the other hand, results were invariably negative for nitrate reduction, pyrazinamidase, pyrolidonyl arylamidase and urease reactions, gelatin hydrolysis, and xylose and mannitol fermentations. Results were invariably positive for alkaline phosphatase, β-glucuronidase reactions, and glucose and lactose fermentations.

Among the 15 *A. hippocoleae* cases reported here, there is a significant number of breeds (n = 8) and horse breeding farms (n = 11), as well as an absence of apparent signs of disease in the mares and a considerable degree of phenotypic and molecular diversity in the *A. hippocoleae* strains highlighted. Our study therefore indicates that the prevalence of *A. hippocoleae*, which can be considered a commensal bacterium in horses—particularly in the genital tract—is greater than thought. The role this species plays as an opportunistic pathogen in horses remains unclear, but the observations in this study raise the question as to whether genetic differences among strains might distinguish potentially pathogenic from commensal strains. *A. hippocoleae* CIP 106850$^T$, possibly responsible for vaginal discharge in a mare (Hoyles et al., 2002), and strain ID 13, isolated from a mare one month after an abortion, are very similar since our results indicate that they diverge only by β-glucosidase reaction; however, the *A. hippocoleae* strain responsible for placentitis and stillbirth in a mare (Bemis et al., 2008) seems more divergent, probably being closer to group B as presented in this work. Our study likewise suggests that the presence of *A. hippocoleae* in the genital tract of horses is sex-specific. Indeed, only mares were affected despite a context of case reports from official CEM tests theoretically more favourable to detecting cases in stallions; this is consistent with cases in the literature when the sex of the animal was known (Hoyles et al., 2002; Bemis et al., 2008; Wickhorst et al., 2017). Nevertheless, this sex-specific isolation of *A. hippocoleae* is questionable because the CEM tests performed in 2017 by LABOCEA35 presented a biased sex-ratio of 91% of mares. A national prevalence survey would be required to estimate the frequency of *A. hippocoleae* carriage in mares and stallions and verify the presence of *A. hippocoleae* outside the French region of Brittany, which is the only one found to be affected in the current study, probably because the isolates were recovered from a single field laboratory in this region. Moreover, systematic studies probably including experimental infections would be required to clarify the pathogenic role of *A. hippocoleae*.

## Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with any other people or organisations that could inappropriately influence or bias the content of the paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2018.11.026.

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