ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 3, 409-413
www.aaem.pl

Aeromonas hydrophila subsp. *dhakensis* – a causative agent of gastroenteritis imported into the Czech Republic

Ivo Sedláček[1], Eva Krejčí[2], Anna Andělová[2], Michaela Sedláčková[1], Iva Porazilová[2], Pavla Holochová[1]

[1] *Czech Collection of Microorganisms, Department of Experimental Biology, Masaryk University, Brno, Czech Republic*
[2] *Institute of Public Health, Centre for Clinical Laboratories, Ostrava, Czech Republic*

Sedláček I, Krejčí E, Andělová A, Sedláčková M, Porazilová I, Holochová P. *Aeromonas hydrophila* subsp. *dhakensis* – a causative agent of gastroenteritis imported into the Czech Republic. Ann Agric Environ Med. 2012; 19 (3): 409-413.

Abstract

Out of the twenty-one *A. hydrophila* complex isolates obtained during a routine examination of human diarrhoeal faeces, two *A. hydrophila* subsp. *dhakensis* isolates (P1097 = CCM 7329 and P1165) were successfully identified by ribotyping. The correct taxonomic position of the *A. hydrophila* subsp. *dhakensis* CCM 7329 was verified by *cpn60* sequencing (GeneBank accession number HM536193). The remaining *A. hydrophila* complex isolates were identified as *A. hydrophila* subsp. *hydrophila*. The ability of biochemical tests and fatty acid methyl ester analysis to reliably discern both *A. hydrophila* subsp. *dhakensis* and *A. hydrophila* subsp. *hydrophila* was limited. In contrast to the *A. hydrophila* subsp. *hydrophila*, the faecal isolates of *A. hydrophila* subsp. *dhakensis* did not produce acid from arbutin. When compared in a two-dimensional plot, the *A. hydrophila* subsp. *dhakensis* faecal isolates contained higher amounts of the two minor fatty acids $C_{13:0}$ and $C_{17:1}$ ω8c than the *A. hydrophila* subsp. *hydrophila* reference strain. This is the first detected occurrence of the less frequent *A. hydrophila* subsp. *dhakensis* in our region and ribotyping was proved as a suitable method for the identification of *A. hydrophila* subsp. *dhakensis*.

Key words

Aeromonas; Identification; Ribotyping; Fatty acids; *cpn60* sequencing

INTRODUCTION

Aeromonads are ubiquitous micro-organisms, they occur in both fresh and saline waters and in soil [1]. Members of the genus *Aeromonas* are mentioned as the causative agents of intestinal and extra-intestinal infections in both immunocompetent and immunocompromised patients [2]. Clinical *Aeromonas* strains are known to be isolated mainly from gastroenteritis cases. Wound infections or septicaemia due to aeromonads have also been reported [3, 4]. Nowadays, owing to many changes in the *Aeromonas* taxonomy, there are 25 valid species of genus *Aeromonas* [5, 6]. The occurrence of a new species or subspecies increases also in human clinical material. Besides the traditional species of *Aeromonas hydrophila*, *Aeromonas caviae* or *Aeromonas veronii* biovar Sobria, other species, such as *Aeromonas jandaei*, *Aeromonas veronii* biovar Veronii or *Aeromonas schubertii*, were recognized as human pathogens [7]. Among the rare species causing gastroenteritis, *Aeromonas media*, *Aeromonas encheleia* and *Aeromonas bestiarum* were included as well [8].

Changes in taxonomy affected also the *A. hydrophila* species, commonly being isolated from clinical samples, animals and the environment [6, 9, 10]. From 2002, two subspecies of *A. hydrophila* exist: *A. hydrophila* subsp. *dhakensis* and *A. hydrophila* subsp. *hydrophila* [9]. Description of *A. hydrophila* subsp. *dhakensis* was based on isolates which came mainly from gastroenteritis cases from Dhaka, Bangladesh. In 2003 the isolates from septicaemic farmed frogs from Thailand were described as the last, third subspecies *A. hydrophila* subsp. *ranae* [10].

Only two subspecies, *A. hydrophila* subsp. *dhakensis* and *A. hydrophila* subsp. *hydrophila*, occurred in human clinical material and belonged to the same phylogenetic group [9].

Biochemical identification of aeromonads to the species level is difficult [11, 12, 13]. At present it is recommended that routine isolates recovered from uncomplicated cases of gastroenteritis should be identified at least as belonging to one of the "complexes" – such as *A. hydrophila* complex (comprises *A. hydrophila*, *A. bestiarum*, *A. popoffii, A. salmonicida*), *A. caviae* complex (*A. caviae*, *A. media*, *A. eucrenophila*) or *A. sobria* complex (*A. veronii* biovar Sobria, *A. jandaei*, *A. schubertii*, *A. enteropelogenes*) [9, 10, 11, 12].

Faecal isolates that are classified as *A. hydrophila* complex are regularly regarded as the *A. hydrophila* subsp. *hydrophila*. The aim of our study was to prove if *A. hydrophila* subsp. *dhakensis* can also be isolated from human faeces and thus be involved in diarrhoeal diseases in our region. Because biochemical identification is still frequently used in routine clinical laboratories, we tried to prove the reliability of biochemical tests to discern *A. hydrophila* subspecies. For confirmation of biotyping results, ribotyping, cellular fatty acid analysis and *cpn60* sequencing were chosen.

Address for correspondence: Ivo Sedláček, Czech Collection of Microorganisms, Department of Experimental Biology, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic.
E-mail: ivo@sci.muni.cz

Received: 18 September 2011; accepted: 13 June 2012

MATERIALS AND METHODS

Bacterial strains

The group of intestinal aeromonads was isolated during a routine survey of human faeces samples from cases of acute diarrhoea in 2003 and 2004. Faeces samples were inoculated on MacConkey, Deoxycholate citrate agar (both Merck) and in addition to select aeromonads from other intestinal flora on Ampicillin Blood Agar (Columbia Blood Agar Base, Merck, with 10% of sheep's blood and 20 mg/L of ampicillin) and Aeromonas agar (Bile Salt Irgasan Brilliant Green Agar) [14]. Faeces samples were cultivated at 36°C for 18-24 hours and typical colonies were isolated. Oxidase-positive, glucose-fermenting Gram-negative rods, resistant to vibriostatic agent O/129 (150 μg, Oxoid) were considered as presumptive *Aeromonas* spp. and decided upon for phenotypic identification to the species level.

Twenty-one faecal isolates, which were classified as *A. hydrophila* complex, were further studied. Stock cultures were stored on glass beads at -70°C in Nutrient broth (Merck) supplemented with glycerol 15% v/v. The following reference cultures were obtained from the Czech Collection of Microorganisms (www.sci.muni.cz/ccm): *A. hydrophila* subsp. *hydrophila* CCM 7232^T, CCM 2280 and CCM 4528, *A. hydrophila* subsp. *dhakensis* CCM 7146^T, *A. hydrophila* subsp. *ranae* CCM 7147^T, *A. bestiarum* CCM 4707^T and *A. popoffii* CCM 4708^T.

Phenotyping

A 10% blood agar (Columbia Blood Agar Base, Merck) was used for cultivation and also to prove beta-haemolysis. Strains were grown at 36°C for 18-24 hours. The biochemical tests were incubated at 36°C and the final results were evaluated after 24-48 hours. All cultivations were carried out in an ambient atmosphere. Both hydrolysis of esculine and gluconate utilization were performed as previously described [15]. Hydrolysis of gelatine and Tween 80 were performed by Páčová and Kocur [16]. The acidification of L-arabinose, arbutin, D-mannose and salicin (all Sigma-Aldrich) were tested in OF basal medium (Difco) with the 1% final concentration of each carbohydrate. Motility was shown in the motility test medium (BBL Becton Dickinson). The ability to grow at 42°C was shown on the MacConkey agar. The production of elastase was tested on the brain heart infusion agar (Oxoid) supplemented with elastin-congo red (3 g/L, Sigma-Aldrich). A DNase agar (Oxoid) supplemented with 0.5 ml/L toluidin blue solution (1 g/L of toluidin blue was dissolved in 40% ethanol) was used to show DNase production. To visualize positive reaction, 10% HCl was added. The production of acid and gas from glucose was tested in Hottinger's broth (tryptose 10 g/L, NaCl 5 g/L, K_2HPO_4 1 g/L; pH 7.4). Bromthymol blue solution (4 ml/L) and glucose (10 g/L) were added to the Hottinger's broth to complete the medium. The commercial kit of microtests ENTEROtest24 (Pliva-Lachema Diagnostika) was used according to the manufacturer's instructions and results were evaluated by TNW software (version 6.0).

Ribotyping

Ribotyping (21 faecal isolates from *A. hydrophila* complex and 7 reference cultures) was performed using *Eco*RI and *Pvu*II restriction enzymes (Bio Labs) and a DNA probe complementary to 16S and 23S rRNA (Roche Diagnostics) according to Švec et al. [17]. Cluster analysis of ribotype profiles was performed using the BioNumerics v. 6.5 software (Applied-Maths). The dendrogram was calculated with Dice coefficients with the unweighted pair group method using arithmetic averages (UPGMA). An optimisation value of 0.5% was automatically determined by the BioNumerics software.

cpn60 sequencing

DNA isolation was performed by phenol-chloroform DNA extraction [17]. The PCR mix and primer sequences were used according to Miñana-Galbis et al. [8]. The thermal cycling conditions were 30 cycles of denaturation at 94°C for 30 s (180 s for the first cycle), annealing at 55°C for 30 s, and polymerization at 72°C for 60 s (300 s for the last cycle). The 555-bp *cpn60* gene sequences were purified using the High Pure PCR product purification kit (Roche Diagnostics) and sequencing was performed by Eurofins MWG Operon (Ebersberg, Germany). The multiple alignments were obtained by using CLUSTAL W software [18]. The phylogenetic tree construction was performed using MEGA4 software, version 4 [19], through neighbour-joining [20]. The reference *cpn60* gene sequences of the *Aeromonas* species used in Miñana-Galbis et al. [8] were downloaded from GenBank.

Whole-cell fatty acid analysis

Cellular fatty acid analysis was done using Microbial Identification System [MIS] Sherlock (MIDI, Inc., Newark, DE, USA). Strains for whole-cell fatty acid analysis were grown on Trypticase soy broth (BBL, Becton Dickinson) solidified with agar (15 g/L, Difco, Becton Dickinson) for 48 hours at 28°C in ambient atmosphere. One loop of freshly growing culture was transferred into the tubes with Teflon-lined screw caps, then converted to methyl esters by means of heated alkaline hydrolysis and extracted. Saponification, methylation and extraction of the cellular fatty acids were performed according to the operating manual of the MIS Sherlock [21]. The samples were analysed on a 25 m by 0.2 mm phenyl-methyl siloxan capillary column using an HP 6890 gas chromatograph equipped with a flame-ionisation detector. Peaks of gained fatty acid methyl ester (FAME) profiles were named with the aid of available MIS Sherlock software. To compare the obtained FAME profiles, a cluster analysis was used to create the dendrogram, as well as the two-dimensional plot cluster analysis technique, which uses principal component analysis to separate groups of samples in *n*-dimensional spaces to find relationships among fatty acid profiles (Library Generation Software) [22].

RESULTS AND DISCUSSION

Twenty one aeromonads isolated from human faeces were classified into the *A. hydrophila* complex level according to the positive results of the following conventional tests: hydrolysis of esculine, Voges-Proskauer test and production of gas from glucose [12]. All these strains were further recognized as *A. hydrophila* species by positive acid production from L-arabinose and salicin and negative acid production from sorbitol [11], as well as by ENTEROtest 24 kit results and proposed for further identification to the subspecies level.

We went on to discern *A. hydrophila* subsp. *hydrophila* and *A. hydrophila* subsp. *dhakensis* also according to their

phenotype and thus found differences in phenotypic results useful for routine laboratories. As we have found, none of the thirty-six phenotypic test results were unique for any of the above-named subspecies. Interestingly, almost all *A. hydrophila* subsp. *hydrophila* strains produced acid from arbutin, whereas *A. hydrophila* subsp. *dhakensis* strains did not; the results of acid production from arbutin have never been mentioned previously [9]. Only one *A. hydrophila* subsp. *hydrophila* faecal isolate was negative for arbutin (eighteen *A. hydrophila* subsp. *hydrophila* faecal isolates and all *A. hydrophila* subsp. *hydrophila* reference strain produced acid from arbutin). Therefore, the inability to produce acid from arbutin seems to distinguish *A. hydrophila* subsp. *dhakensis* from *A. hydrophila* subsp. *hydrophila*.

Production of acid from L-arabinose is one of the four key characteristics useful for the classification to the *Aeromonas* spp. As mentioned before [9], also in our study *A. hydrophila* subsp. *dhakensis* strains did not produce acid from L-arabinose. In contrast to the previous results from Huys et al. [9], we have observed three *A. hydrophila* subsp. *hydrophila* strains which did not produce acid from L-arabinose (P940, P954, CCM 7232^T) and the reliability of the test remains unclear.

Among the methods used for separation of faecal isolates from *A. hydrophila* complex, ribotyping appeared to be the most appropriate tool. Using the *Eco*RI restriction enzyme, the dendrogram of 28 ribotyping patterns showed two major clusters at a similarity of approximately 55%; reference strains *A. bestiarum* CCM 4707^T and *A. popoffii* CCM 4708^T were separated from the others (Fig. 1). Out of the twenty-one *A. hydrophila* complex faecal isolates, two strains P1097 (= CCM 7329) and P1165 were identified as *A. hydrophila* subsp. *dhakensis* based on the cluster analysis of ribotype patterns in comparison with bands of reference culture CCM 7146^T and they represented the first cluster. The remaining nineteen faecal isolates were identified as *A. hydrophila* subsp. *hydrophila* on the basis of ribotyping and they are arranged in the second cluster together with a chosen three reference strains of *A. hydrophila* subsp. *hydrophila* (Fig. 1). This second cluster comprising the majority of analysed strains was divided into several sub-clusters with a similarity of about 70% and the bands were located between 1.80 and 22.00 Kb. No member of *A. hydrophila* subsp. *ranae* was proved in the analysed group of faecal aeromonads. All faecal isolates and reference cultures from *A. hydrophila* complex showed very diverse ribotype patterns and thus ribotyping ensured the sufficient differentiation of these taxa. The *Eco*RI ribotype patterns of both *A. hydrophila* subsp. *dhakensis* faecal strains were identical. About a 1.0 Kb restriction fragment was observed in all *A. hydrophila* subsp. *dhakensis* ribotype patterns (Fig. 1), but was absent among ribotypes of all analyzed *A. hydrophila* subsp. *hydrophila* strains. Otherwise, *Pvu*II ribotype patterns of analyzed *A. hydrophila* subsp. *dhakensis* cultures (Fig. 2) showed ten bands placed between approximately 2.1 and 15.0 Kb. Restriction fragment profiles of faecal isolates *A. hydrophila*

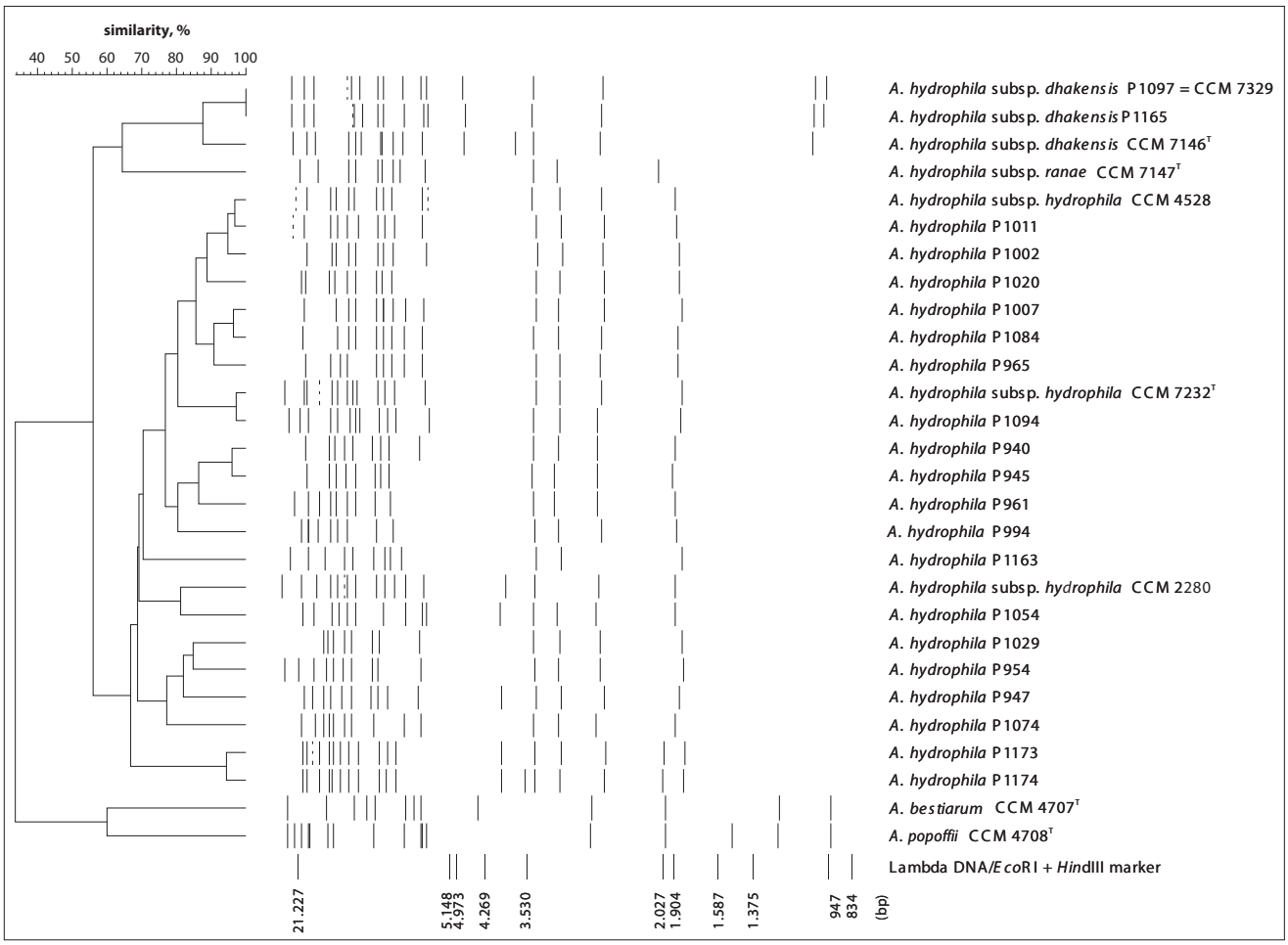

Figure 1. Dendrogram based on cluster analysis of ribotype patterns of *Aeromonas hydrophila* complex obtained with *Eco*RI and a probe complementary to 16S and 23S rRNA of *Escherichia coli*

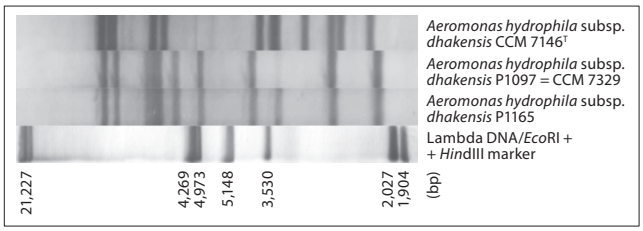

Figure 2. *PvuII* ribotype patterns of analysed *Aeromonas hydrophila* subsp. *dhakensis* cultures

subsp. *dhakensis* (P1097 = CCM 7329 and P1165) obtained with *Pvu*II endonuclease were slightly different from the profile of type strain *A. hydrophila* subsp. *dhakensis* CCM 7146^T. Ribotyping demonstrated a close relationship among *A. hydrophila* subsp. *dhakensis* cultures with coincident heterogeneity between type strain and faecal strains.

The DNA sequencing and phylogenetic tree construction was performed to verify the efficient of A. *hydrophila* subsp. *dhakensis* identification by the ribotyping method. The 555-bp *cpn60* gene sequence of the isolate P1097 (= CCM 7329), GeneBank accession number HM536193, was compared with the reference *cpn60* gene sequences of the *Aeromonas* spp. in accordance with Miñana-Galbis et al. [8]. The method demonstrated a close relation between faecal isolate P1097 (= CCM 7329) and type strain of A. *hydrophila* subsp. *dhakensis* (EU306806) on a 98.7% similarity (Fig. 3). The discrimination based on *cpn60* sequence divergence of *Aeromonas* species was established, the intraspecies divergence rates were ≤ 3.5%, while interspecies divergence rates ranged from 3.7 to 16.9% [8]. As the dendrogram shows, the A. *hydrophila* subsp. *dhakensis* sequence was clearly separated from A. *hydrophila* subsp. *hydrophila* and A. *hydrophila* subsp. *ranae* sequences.

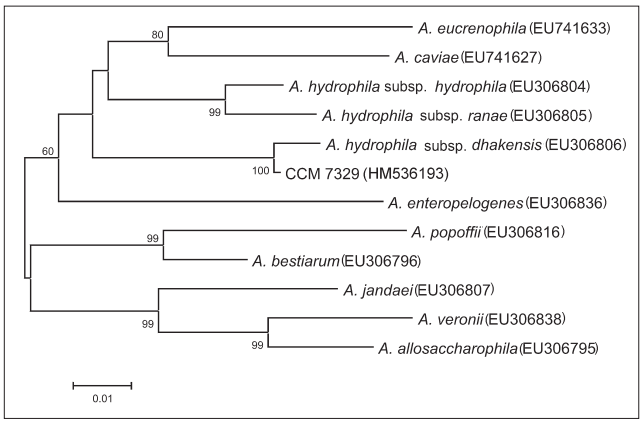

Figure 3. Dendrogram showing the genetic relationship of the representative *Aeromonas* species based on the 555-bp *cpn60* gene sequence. GenBank accession numbers are indicated in parentheses. The phylogenetic tree was reconstructed applying the neighbour-joining method in combination with the Jukes Cantor model. The tree topology was evaluated by performing 1000 bootstrap runs (> 50% values are shown). A bar indicates 10% estimated sequence divergence

Cellular fatty acid composition has been successfully used for the differentiation of *Aeromonas* species before [23]. As expected, the high relative amounts of the saturated fatty acid $C_{16:0}$ were detected in all twenty-one faecal isolates, which was described as a typical feature for *A. hydrophila* species [23]. The FAME profiles of *A. hydrophila* subsp. *dhakensis* faecal isolates were recognized as the most similar to the *A. hydrophila* subsp. *dhakensis* type strain (data not shown). The classification of an unknown *A. hydrophila* isolate as the presumptive *A. hydrophila* subsp. *dhakensis* according to the fatty acid analysis results was tested using a two-dimensional plot. The position in the plot of each isolate or strain is based on the percentage of the two minor fatty acids $C_{13:0}$ and $C_{17:1}$ ω8c (Fig. 4). All three type strains (*A. hydrophila* subsp. *hydrophila*, *A. hydrophila* subsp. *dhakensis* and *A. hydrophila* sups. *ranae*) were well-distinguished from each other. Both *A. hydrophila* subsp. *dhakensis* faecal isolates were positioned beside the *A. hydrophila* subsp. *dhakensis* type strain. Almost all *A. hydrophila* subsp. *hydrophila* faecal isolates (excepting P940, P947, P954) clustered together with the *A. hydrophila* subsp. *hydrophila* type strain. Comparing *A. hydrophila* subsp. *hydrophila* and *A. hydrophila* subsp. *dhakensis* the higher amount of two named minor fatty acids – $C_{13:0}$ and $C_{17:1}$ ω8c – can signalize the possible presence of *A. hydrophila* subsp. *dhakensis* isolate.

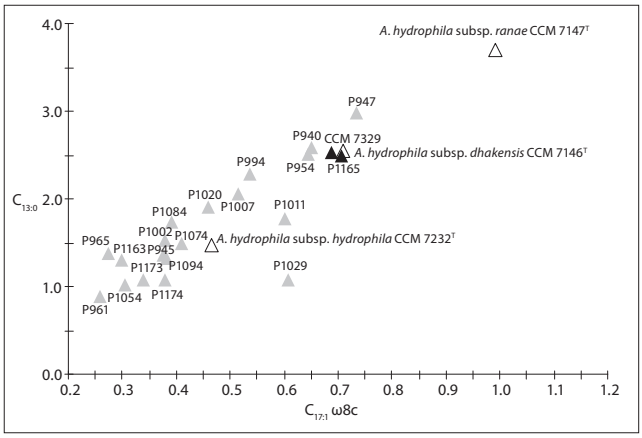

Figure 4. The two-dimensional plot showing type strains of the three subspecies of *A. hydrophila* and *A. hydrophila* complex faecal isolates. The position in the plot is based on the percentage of the two minor fatty acids $C_{13:0}$ and $C_{17:1}$ ω8c.

This is the first detected occurrence of the *A. hydrophila* subsp. *dhakensis* in our region proved by ribotyping and *cpn60* sequencing results. Up to now, all known *A. hydrophila* subsp. *dhakensis* isolates came from Dhaka, Bangladesh. Isolates originated mainly from children who suffered from watery diarrhoea; the majority of isolates demonstrated strong haemolytic and cytotoxic activity. Rarely, some isolates came from healthy control samples or from the surface water [24]. We concluded that isolated *A. hydrophila* subsp. *dhakensis* occurred in our region most probably as an imported case of the infection: after returning from Egypt, a 25-year old man suffered from the acute profuse diarrhoea for ten days. To examine the reason for the infection, two faecal samples in an interval of fourteen days were examined. Repeatedly, *A. hydrophila* subsp. *dhakensis* was determined as the sole causative agent (isolate P1097 = CCM 7329 and isolate P1165) – no other diarrhoeal pathogens (such as *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica* or *Campylobacter* spp.) were isolated. The patient recovered after symptomatic treatment with intestinal anti-infective agents. Subsequent epidemiological investigation by ribotyping proved that both *A. hydrophila* subsp. *dhakensis* faecal isolates represent two isolates of one strain. Isolate P1097 was deposited in the Czech Collection of Microorganisms as CCM 7329.

To our knowledge, no other references regarding *A. hydrophila* subsp. *dhakensis* isolates connected with diarrhoeal disease have been published with the exception of the subspecies description given by Huys et al. [9]. The

faecal *Aeromonas*-isolates come typically from very young children or old people, isolation of aeromonads as a causative agent of the gastroenteritis from a young man is exceptional. It is known that aeromonads do cause gastroenteritis [25], but, on the other hand, strains of the genus *Aeromonas* occur in the faeces of people both with and without diarrhoea [26].

CONCLUSIONS

Our survey indicates that *A. hydrophila* subsp. *dhakensis* can occur in our region in central Europe as an imported illness. For a better knowledge of which taxa are responsible for serious cases of acute diarrhoea, a differentiation method useful in clinical laboratories among *A. hydrophila* subsp. *hydrophila* and *A. hydrophila* subsp. *dhakensis* will be helpful [27]. *A. hydrophila* strains which did not produce acid from arbutin or contain higher amounts of $C_{13:0}$ and $C_{17:1}$ ω8c fatty acids (comparing with *A. hydrophila* subsp. *hydrophila* reference strains) can be marked as presumptive *A. hydrophila* subsp. *dhakensis*. Members of *A. hydrophila* subsp. *dhakensis* could be easily identified by other discriminative methods such as realized ribotyping (*Eco*RI restriction enzyme) or *cpn60* gene sequencing.

Acknowledgements

This work was supported by the long-term research programme no. MSM0021622416 of the Ministry of Education, Youth and Sports of the Czech Republic

REFERENCES

1. Sedláček I, Jakšl V, Přepechalová H. Identification of aeromonads from water sources. Epidemiol Mikrobiol Imunol. 1994; 43: 61-66 (in Czech).
2. Janda JM, Guthertz LS, Kokka RP, Shimada T. *Aeromonas* species in septicaemia: laboratory characteristics and clinical observations. Clin Infect Dis. 1994; 19: 77-83.
3. Grobusch MP, Gobels K, Teichmann D. Cellulitis and septicaemia by *Aeromonas hydrophila* acquired at home. Infection. 2001; 29: 109-110.
4. Janda JM. Recent advances in the study of the taxonomy, pathogenicity, and infectious syndromes associated with genus *Aeromonas*. Clin Microbiol Rev. 1991; 4: 397-410.
5. Euzéby JP. List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. Int J Syst Bacteriol. 1997; 47: 590-592. (List of Prokaryotic names with Standing in Nomenclature. (http://www.bacterio.net, access: 2011.08.10).
6. Martin-Carnahan A, Joseph SW. Genus I. *Aeromonas*. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT. Bergey's Manual of Systematic Bacteriology. Second edition. Volume Two. The *Proteobacteria*. Part B The *Gammaproteobacteria*. USA, Springer, 2005. p. 557-578.
7. Miyake M, Iga K, Izumi C, Miyagawa A, Kobashi Y, Konishi T. Rapidly progressive pneumonia due to *A. hydrophila* shortly after near-drowning. Intern Med. 2000; 39: 1128-1130.
8. Miñana-Galbis D, Urbizu-Serrano A, Farfan M, Fusté MC, Loren JG. Phylogenetic analysis and identification of *Aeromonas* species based on sequencing of the *cpn60* universal target. Int J Syst Evol Microbiol. 2009; 59: 1976-1983.
9. Huys G, Kämpfer P, Albert MJ, Kühn I, Denys R, Swings J. *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov., isolated from children with diarrhoea in Bangladesh, and extended description of *Aeromonas hydrophila* subsp. *hydrophila* (Chester 1901) Stainer 1943. Int J Syst Evol Microbiol. 2002; 52: 705-712.
10. Huys G, Pearson M, Kämpfer P, Denys R, Cnockaert M, Inglis V, Swings J. *Aeromonas hydrophila* subsp. *ranae* subsp. nov., isolated from septicaemic farmed frogs in Thailand. Int J. Syst Evol Microbiol. 2003; 53: 885-891.
11. Abbott SL. Aeromonas. In: Murray PR, Baron EJ, Jorgenson JH, Pfaller MA, Yolken RH. Manual of Clinical Microbiology, 8th edition. Washington, D.C., ASM Press, 2003. p. 701-705.
12. Abbott SL, Cheung WKW, Janda JM. The genus *Aeromonas*: biochemical characteristics, atypical reactions and phenotypic identification schemes. J Clin Microbiol. 2003; 41: 2348-2357.
13. Abbott SL, Seli LS, Catino M Jr., Hartley MA, Janda JM. Misidentification of unusual *Aeromonas* species as members of genus *Vibrio*: a continuing problem. J Clin Microbiol. 1998; 36: 1103-1104.
14. Andělová A, Porazilová I, Krejčí E. Aeromonas agar is a useful selective medium for isolating aeromonads from faecal samples. J Med Microbiol. 2006; 55: 1605-1606.
15. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. Cambridge, University Press, 1974. p 150-171.
16. Páčová Z, Kocur M. New medium for detection of esterase and gelatinase activity. Zbl Bakt Hyg A. 1984; 258: 69-73.
17. Švec P, Sedláček I, Pantůček R, Devriese LA, Doškař J. Evaluation of ribotyping for characterization and identification of *Enterococcus haemoperoxidus* and *Enterococcus moraviensis* strains. FEMS Microbiol Lett. 2001; 203: 23-27.
18. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22: 4673-4680.
19. Tamura K, Dudley J, Nei M, Kumar S. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 4. Mol Biol Evol. 2007; 24: 1596-1599.
20. Saitou N, Nei M. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4: 406-425.
21. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI technical note 101. MIDI, Newark, DE, USA 2001. (http://www.midi-inc.com/media/pdfs/TechNote_101.pdf).
22. MIDI, Inc. Library Generation Software user's manual, version 4.5. MIDI, Inc., Newark, DE, USA 2002.
23. Huys G, Vancanneyt M, Coopman R, Janssen P, Falsen E, Altwegg M, Kersters K. Cellular fatty acid composition as a chemotaxonomic marker for differentiation of phenospecies and hybridization groups in the genus *Aeromonas*. Int J Syst Bacteriol. 1994; 44: 651-658.
24. Kühn I, Albert MJ, Ansaruzzaman M, Bhuiyan NA, Alabi SA, Islam MS, Neogi PK, Huys G, Janssen P, Kersters K, Möllby R. Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls, and from surface water in Bangladesh. J Clin Microbiol. 1997; 35: 369-373.
25. Albert M, Faruque ASG, Faruque SM, Sack RB, Mahalanabis D. Case-control study of enteropathogens associated with childhood diarrhoea in Dhaka, Bangladesh. J Clin Microbiol. 1999; 37: 3458-3464.
26. Farmer III JJ, Arduino MJ, Hickman-Brenner FW. The Genera *Aeromonas* and *Plesiomonas*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E. The Prokaryotes, A Handbook on the Biology of Bacteria: An Evolving Electronic Resource for the Microbiological Community. 3rd edition. Volume 6: Proteobacteria: Gamma subclass. New York, Springer-Verlag, 2006. p. 564-596. (http://link.springer-ny.com/link/service/books/10125/).
27. Ørmen Ø, Granum PE, Lassen J, Figueras MJ. Lack of agreement between biochemical and genetic identification of *Aeromonas* spp. APMIS. 2005; 113: 203-207.