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# Original paper

# **Online** Advanced Bacterial Identification Software, an Original Tool for Phenotypic Bacterial Identification

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Abstract The objective of the study is to present and validate an original online Advanced Bacterial Identification Software, ABIS, by comparison to a commercially available, standardized identification system, API strips and apiweb<sup>™</sup> bioMerieux software.

Methods and results: presentation of ABIS online software, phenotypic bacterial identification of 16 reference strains and 123 wild isolates by ABIS and apiweb TM bioMerieux software and comparative analysis of results. Closed results were obtained (same taxa) for reference and wild strains of Enterobacteriaceae, Pasteurellaceae, Bacillaceae, Lactobacillaceae, Staphylococcaceae, Streptococcaceae, and other.

Conclusions: Apiweb<sup>™</sup> confirmed the results of ABIS, overall, average identification percent for ABIS being 91.8% and 90.4% for apiweb TM. ABIS online is a powerful tool for microbiology lab and the Encyclopedia connection provides essential information about the ecological significance, pathology and other features of the identified strains.

Keywords phenotypic bacterial identification, original online software

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### Introduction

In the fields of applied bacteriology, human and veterinary clinic, phytopathology and also in environmental microbiology, bacterial strains from various sources are routinely isolated and phenotypically identified. Beside a properly equipped laboratory, this activity, on the edge between science, technique, craftsmanship and art, requires specific culture media, reagents, experienced specialists in bacteriology, and a bacterial identification system. The identification systems may be the manuals of bacteriology (Borriello, 2006; Buiuc, 2009; Gordon, 1973; Dworkin, 2006), Bergey's Manuals of Determinative (Buchanan, 1975; Holt, 1994) and Systematic (Vos, 2009; Goodfellow, 2012; Garitty, 2004) Bacteriology, review articles on specific bacteria categories (Funke, 1997), commercial systems like apiweb<sup>™</sup> (bioMerieux), Biolog, and online systems. Each of these systems has its limitations, well-known by the specialists (no interactivity, no updating for the newly described taxa, constraining the user to a predefined set of tests, no instant connection to a database of ecological significance and pathology of the isolated strains). To tackle these problems we created ABIS online - Advanced Bacterial Identification Software (http://www.tgw1916.net), a laboratory tool for bacterial identification, based on morphology, biochemical characters, cultural characteristics, ecology and pathogenicity data. The program allows a great flexibility in choosing biochemical tests and it is an alternative to commercial systems, code-books or identification tables. It is a free software, available on World Wide Web since 2007, being increasingly used by students, young researches, biologists and human and veterinary clinicians.

The aim of the study was to present and validate ABIS software by comparison to a commercially available, standardized identification system.

#### **Materials and Methods**

ABIS online (*ABIS online - bacterial identification software* version 12, http://www.tgw1916.net) is a free bacterial identification software available on World Wide Web.

The identification scheme is based on the comparison between the morphological and biochemical character values of the isolated strain and standard values of taxa contained in the database. Thirteen databases are currently available, covering areas of *Enterobacteriaceae*, *Pasteurellaceae*, *Campylobacteraceae*, *Bacillaceae*, *Lactobacillaceae*, *Staphylococcaceae*, *Streptococcaceae*, *Clostridium*, *Vibrio* and *Aeromonas*, *Listeriaceae*, *Neisseriaceae*, *Chromobacterium*, *Corynebacterium*, and Non-fermenters (fig. 1).

The databases were created taking into consideration the characteristics of type strains of each species, the characteristics of strains existing in international collections and rigorously characterized strains, generally described in Bergey's Manual of Systematic Bacteriology (7-9), Bergey's Manual of Determinative Bacteriology (5, 6), International Journal of Systematic and Evolutionary Microbiology, Topley & Wilson's Manual (1), as well as review articles (10).

ABIS is not an annex of an identification kit, so the type and number of the required tests are not limited by a pattern and can vary by lab availabilities (fig. 2). Depending on the number of the introduced tests, ABIS calculates the percentage of similarity with taxa from the database and the accuracy of identification (fig. 3). The quality of results depends on the number of tests entered by the user. At least 8 tests are required, and the maximum depends on laboratory capacity and database limitations. ABIS databases contain an average of 40-50 tests from which the user can choose.

The most important feature of ABIS is that it allows the user to instantly access data from Encyclopedia about morpho-biochemical characters, cultural characteristics, ecology and pathogenicity of the identified taxa, leaving the final decision to the human factor, not to the machine.

The morphological and biochemical characters of 16 reference strains and 123 wild isolates were tested by ABIS online and apiweb<sup>™</sup> software (bioMerieux) using all laboratory available tests (classical tests and API bioMerieux strips). The reference strains were derived from ROMVAC Company and Cantacuzino Institute Bacterial Collections. The wild strains were isolated from various human, animal and environmental sources: milk, urine, pus, vaginal secretion, ear secretion, skin, internal organs from deceased animals (liver, kidney, lungs, bone marrow, salpinx), yolk sac, sanitation tests and animal feed.

Commercially available tests (Gram staining, oxidase, Simmons citrate, Triple Sugars Iron -TSI-, Motility Indole Urea -MIU-, hemolysis on sheep blood agar), together with commercially available API bioMerieux kits (API 20E, API 20 NE, API Staph, API Strep, API Campy, API 20A and API 50CHB/CHL) were used for identification.



Figure 1. ABIS online Main Menu – Selecting databases.

Auto	mode 🔹					1	ack Refresh	Target Tests Com	inue
				Lactob	acillus datab	ase			
		Posthe	Negative	Unknown			Positive N	ngatiwe Unikasaam	
12	Spores production					Galactose			
51	Growth at 15°C				12.	Gluconate			
	Growth at 45°C				41.	Glucose			
m	Motility				100	Glycerol			
					10	Inositol			
					100	Inulin			
**	Catalase				222	Lactose			
	Oxidase				27.6	Maltose		• •	
++=	Indole production					Mannitol			
	H <sub>2</sub> S production				10.1	Mannose			
÷.	Nitrates reduction				47.	Melezitose		/	
	Gelatin hydrolysis					Melibiose		• • (	
-21	Arginine hydrolysis					Raffinose		· · La.	
	Gas from Glucose				22.	Rhamnose			
						Ribose		· · ·	
					222	Salicin		0/0/	A CONTRACTOR
Ferm	entation of:					Sorbitol			
	Amygdalin					Sorbose			
**	Arabinose				.31	Starch		• •	111
***	Cellobiose				.÷.	Sucrose			
	Esculin				10.	Trehalose			

Figure 2. Input page of ABIS online.



Figure 3. Results page of ABIS online.

#### **Results and discussion**

Table 1 shows the tested reference strains and results of comparative identification with ABIS online and apiweb<sup>™</sup> software.

Very closed results (same taxa, with low difference,  $\leq$  10%, in id%) were obtained for reference strains in comparative identification with ABIS online and apiweb<sup>TM</sup> software.

Table 2 shows the tested wild strains together with their source of isolation and results of comparative identification with ABIS online and apiweb<sup>TM</sup> software.

Comparable results (same taxa), but with important difference in the average id% for *Staphylococcus* spp. ( $\leq$  48.3%), *Streptococcus* spp. ( $\leq$  29%) and *Stenotrophomonas* 

*maltophilia* (46%), were obtained for wild strains in comparative identification with ABIS online and apiweb<sup>TM</sup> software.

Both software programs returned the same taxa with a close identification percent for reference strains. For the *Clostridium tetani* RO-2009 strain, apiweb<sup>TM</sup> returned *Clostridium tetani* identity without an id%.

Comparable results were obtained in ABIS and apiweb<sup>™</sup> bacterial identification systems, both for reference and wild strains. In most cases both software programs returned the same taxa and a close identification percent.

For 16 strains apiweb<sup>TM</sup> considered the profile to be "unacceptable", thus returned a suggested taxon wihout calculating the id% and without validating its results. In all these cases ABIS confirmed apiweb's suggested taxa with a high id%.

#	Strain name and code	ABIS results id % <sup>a</sup>	apiweb™ results id % <sup>b</sup>
1.	<i>Staphylococcus aureus</i> ATCC 25293	Staphylococcus aureus subsp. aureus 99%	Staphylococcus aureus 97.8%
2.	<i>Helicobacter pylori</i> ATCC 43504	Helicobacter pylori 94%	Helicobacter pylori 99.9%
3.	Bacillus thuringiensis NCIMB 9134	Bacillus thuringiensis 93%	<i>Bacillus cereus,</i> possibility of <i>B. thuringiensis</i> 98.9%
4.	<i>Bacillus megaterium</i> NCIMB 8508	Bacillus megaterium 90%	Bacillus megaterium 99.6%
5.	<i>Clostridium tetani</i> RO-2009	Clostridium tetani 97%	Clostridium tetani (no id%)
6.	Salmonella Choleraesuis RO-014	Salmonella spp. 87%	Salmonella choleraesuis 99.7%
7.	Salmonella Typhimurium ATCC 14028	Salmonella spp. 94%	Salmonella spp. 99.9%
8.	Salmonella Typhimurium RO-TM2	Salmonella spp. 89%	Salmonella spp. 99.0%
9.	<i>Proteus hauseri</i> ATCC 13315	Proteus vulgaris / P. hauseri 95%	Proteus vulgaris group 99.8%
10.	<i>Escherichia coli</i> ATCC 8739	Escherichia coli 98%	Escherichia coli 99.9%
11.	<i>Streptococcus mutans</i> ATCC 55677	Streptococcus mutans 98%	Streptococcus mutans 99.9%
12.	<i>Enterococcus faecium</i> NCIMB 10415	Enterococcus faecium 97%	Enterococcus faecium 93.9%
13.	<i>Enterococcus faecium</i> NCIMB 11181	Enterococcus faecium 92%	Enterococcus faecium 99.2%
14.	Pseudomonas aeruginosa ATCC 15442	Pseudomonas aeruginosa 92%	Pseudomonas aeruginosa 98.1%
15.	<i>Lactobacillus plantarum</i> ATCC 8014	Lactobacillus plantarum 91%	Lactobacillus plantarum 1 99.9%
16.	Lactobacillus paracasei CCM 1837	Lactobacillus paracasei subsp. paracasei 94%	Lactobacillus paracasei subsp. paracasei 1/3 48.0/51.0%

Table 1. Comparative reference strains identification between ABIS online and apiweb<sup>™</sup> software

Legend: a- ABIS %id represents the percentage of similarity with taxa from the database, all databases containing a matrix where probabilistic incidence values are allocated for every taxon and their corresponding morpho-biochemical characters; b- apiweb<sup>TM</sup> %id is a probabilistic calculation using bioMerieux own system procedure.

Table 2. Comparative wild strains identification between ABIS online and apiweb<sup>™</sup> software.

#	Source	Nr. of tested strains	ABIS results Average id% <sup>a</sup>	apiweb™ results Average id% <sup>b</sup>
1.	Goat mastitis, chicken arthritis, dog skin, cat skin, cow milk, chicken samples	11	Staphylococcus aureus 94.9%	Staphylococcus aureus 94.8%
2.	Cow milk	1	Staphylococcus chromogenes 93%	Staphylococcus chromogenes 86.7%
3.	Sheep and cow milk, vaccine contaminant	4	Staphylococcus epidermidis 96%	Staphylococcus epidermidis 94.4%
4.	Dog and cat skin	4	Staphylococcus intermedius 96%	Staphylococcus intermedius 62.1%
5.	Dog skin	2	Staphylococcus haemolyticus 91%	Staphylococcus haemolyticus 62%
6.	Dog otitis	1	Staphylococcus schleiferi 93%	Staphylococcus schleiferi 44.7%
7.	Dog skin	1	Staphylococcus simulans 89%	Staphylococcus simulans 96.0%

9. Environment       1       Staphylococcus carnosus 88%       Staphylococcus carnosus (no id%)         10 Human skin lesion pus, goat and sheep milk       3       Streptococcus agalactiae 92.6%       Streptococcus agalactiae 99.7%         11 Sheep intestine       1       Streptococcus dysgalactiae subsp.       Streptococcus dysgalactiae subsp.         12 Swine lungs       2       Streptococcus dysgalactiae subsp.       equisimilis 92.5%         13 Goat milk       1       Streptococcus mutans 83%       Streptococcus mutans 99.7%         14 Rabbit lungs, human throat       2       Streptococcus mutans 83%       Streptococcus mutans 99.7%         15 Pig lungs, pig arthritis       2       Streptococcus mutans 84%       Streptococcus mutans 99.7%         16 Bovine vaginal secretion       1       Enterococcus galactiae subsp.       (alternative: S. oralis 88.3%)         17 Chicken samples, dog skin       4       Enterococcus faecalis 93%       Enterococcus faecalis 99.1%         18 Bovine vaginal secretion, goat kidney       2       Enterococcus lactis, subsp. Lactis 90%       Lactococcus faecium 92%         19 Cow milk       1       Lactococcus lactis, subsp. Lactis 90%       Lactococcus lactis, subsp. lactis 90.4%         20 Sanitation test from swine farm       1       Stenotrophomonas maltophilia 91%       Stenotrophomonas aeruginosa 88.5%       Pseudomonas aeruginosa 98.8%<	8. Goat and c	ow milk	3	Staphylococcus xylosus 95%	Staphylococcus xylosus 98.4%
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20 Sanitation test from swine farm       1       Stenotrophomonas maltophilia 91%       Stenotrophomonas maltophilia 45%         21 Chicken yolk sac, water       2       Pseudomonas aeruginosa 88.5%       Pseudomonas aeruginosa 98.8%         22 Dog urinary infection, cat vaginal secretion       2       Chryseomonas luteola 91%       Chryseomonas luteola 99.8% (no id% for 1 strain)         23 Chicken feces       1       Moraxella lacunata 96%       Moraxella lacunata 85.7%         24 Chicken yolk sac       1       Acinetobacter baumannii / A. calcoaceticus 91%       calcoaceticus 91%         25 Chicken intestine       1       Campylobacter jejuni subsp. jejuni biotype 1 86%       Campylobacter jejuni subsp. jejuni biotype 1 99.4%         26 Chicken salpinx, guinea pig lungs       2       Mannheimia haemolytica 85%       Mannheimia haemolytica 85%	19 Cow milk		1	Lactococcus lactis, subsp. Lactis 90%	Lactococcus lactis, subsp. lactis 90.4%
21 Chicken yolk sac, water       2       Pseudomonas aeruginosa 88.5%       Pseudomonas aeruginosa 98.8%         22 Dog urinary infection, cat vaginal secretion       2       Chryseomonas luteola 91%       Chryseomonas luteola 99.8% (no id% for 1 strain)         23 Chicken feces       1       Moraxella lacunata 96%       Moraxella lacunata 85.7%         24 Chicken yolk sac       1       Acinetobacter baumannii / A. calcoaceticus 91%       calcoaceticus 91%         25 Chicken intestine       1       Campylobacter jejuni subsp. jejuni biotype 1 86%       Campylobacter jejuni subsp. jejuni biotype 1 99.4%         26 Chicken salpinx, guinea pig lungs       2       Mannheimia haemolytica 85% (alternative: M. glucosida 77% - 1 strain)       Pasteurella trehalosi 77.5%	20 Sanitation farm	test from swine	1	Stenotrophomonas maltophilia 91%	Stenotrophomonas maltophilia 45%
22 Dog urinary infection, cat vaginal secretion       2       Chryseomonas luteola 91%       Chryseomonas luteola 99.8% (no id% for 1 strain)         23 Chicken feces       1       Moraxella lacunata 96%       Moraxella lacunata 85.7%         24 Chicken yolk sac       1       Acinetobacter baumannii / A. calcoaceticus 91%       Acinetobacter baumannii / A. calcoaceticus 91%         25 Chicken intestine       1       Campylobacter jejuni subsp. jejuni biotype 1 86%       Campylobacter jejuni subsp. jejuni biotype 1 99.4%         26 Chicken salpinx, guinea pig lungs       2       Mannheimia haemolytica 85% (alternative: M. glucosida 77% - 1 strain)       Mannheimia haemolytica 77.5%	21 Chicken yo	olk sac, water	2	Pseudomonas aeruginosa 88.5%	Pseudomonas aeruginosa 98.8%
23 Chicken feces       1       Moraxella lacunata 96%       Moraxella lacunata 85.7%         24 Chicken yolk sac       1       Acinetobacter baumannii / A. calcoaceticus 91%       Acinetobacter baumannii / A. calcoaceticus 91%         25 Chicken intestine       1       Campylobacter jejuni subsp. jejuni biotype 1 86%       Campylobacter jejuni subsp. jejuni biotype 1 99.4%         26 Chicken salpinx, guinea pig lungs       2       Mannheimia haemolytica 85% (alternative: M. glucosida 77% - 1 strain)       Mannheimia haemolytica 77.5%	22 Dog urinar vaginal sec	y infection, cat pretion	2	Chryseomonas luteola 91%	<i>Chryseomonas luteola</i> 99.8% (no id% for 1 strain)
24 Chicken yolk sac       1       Acinetobacter baumannii / A. calcoaceticus 91%       Acinetobacter baumannii / A. calcoaceticus 91%         25 Chicken intestine       1       Campylobacter jejuni subsp. jejuni biotype 1 86%       Campylobacter jejuni subsp. jejuni biotype 1 99.4%         26 Chicken salpinx, guinea pig lungs       2       Mannheimia haemolytica 85% (alternative: M. glucosida 77% - 1 strain)       Mannheimia haemolytica 77.5%	23 Chicken fe	ces	1	Moraxella lacunata 96%	Moraxella lacunata 85.7%
25 Chicken intestine1Campylobacter jejuni subsp. jejuni biotype 1 86%Campylobacter jejuni subsp. jejuni biotype 1 99.4%26 Chicken salpinx, guinea pig lungs2Mannheimia haemolytica 85% (alternative: M. glucosida 77% - 1 strain)Mannheimia haemolytica 77% - 1 Pasteurella trehalosi 77.5%	24 Chicken yo	olk sac	1	Acinetobacter baumannii / A. calcoaceticus 91%	Acinetobacter baumannii / A. calcoaceticus 91%
26 Chicken salpinx, guinea       2       Mannheimia haemolytica 85%       Mannheimia haemolytica /         pig lungs       (alternative: M. glucosida 77% - 1       Pasteurella trehalosi 77.5%	25 Chicken in	testine	1	Campylobacter jejuni subsp. jejuni biotype 1 86%	Campylobacter jejuni subsp. jejuni biotvpe 1 99.4%
pig lungs (alternative: <i>M. glucosida</i> 77% - 1 <i>Pasteurella trehalosi</i> 77.5% strain)	26 Chicken sa	lpinx, guinea	2	Mannheimia haemolytica 85%	Mannheimia haemolytica /
Stram	pig lungs			(alternative: <i>M. glucosida</i> 77% - 1 strain)	Pasteurella trehalosi 77.5%
27 Swine samples, chicken4Pasteurella multocida 89%Pasteurella multocida 95.0%liver and bone marrow	27 Swine sam liver and b	ples, chicken one marrow	4	Pasteurella multocida 89%	Pasteurella multocida 95.0%
28 Goat vaginal secretion1Vibrio parahaemolyticus 77%Vibrio parahaemolyticus 98.9%	28 Goat vagin	al secretion	1	Vibrio parahaemolyticus 77%	Vibrio parahaemolyticus 98.9%
29 Guinea pig intestine and liver2Aeromonas hydrophila 86%Aeromonas hydrophila 97.9%	29 Guinea pig	intestine and liver	2	Aeromonas hydrophila 86%	Aeromonas hydrophila 97.9%
30 Cow milk1Bacillus licheniformis 85%Bacillus licheniformis 99.9%	30 Cow milk		1	Bacillus licheniformis 85%	Bacillus licheniformis 99.9%
31 Cow milk1Bacillus pumilus 80%Bacillus pumilus 99.9%	31 Cow milk		1	Bacillus pumilus 80%	Bacillus pumilus99.9%
32 Pig and chicken intestine 3 Lactobacillus fermentum 89.6% Lactobacillus fermentum 71.7%	32 Pig and chi	icken intestine	3	Lactobacillus fermentum 89.6%	Lactobacillus fermentum 71.7%
<u>33 Bovine intestine 1 Lactobacillus rhamnosus 84% Lactobacillus rhamnosus 99.9%</u>	33 Bovine inte	estine	1	Lactobacillus rhamnosus 84%	Lactobacillus rhamnosus 99.9%
34 Bovine intestine1Lactobacillus paracasei subsp.Lactobacillus paracasei subsp.paracasei 98%paracasei 98%	34 Bovine inte	estine	I	Lactobacillus paracasei subsp. paracasei 98%	Lactobacillus paracasei subsp. paracasei 98%
35 Vaccine and medical product contaminants       3       Citrobacter freundii 96.5%       Citrobacter freundii 99.8%	35 Vaccine an product con	d medical ntaminants	3	Citrobacter freundii 96.5%	Citrobacter freundii 99.8%
36 Chicken feces3Citrobacter youngae 91.6%Citrobacter youngae 99.6%	36 Chicken fe	ces	3	Citrobacter youngae 91.6%	Citrobacter youngae 99.6%
37 Dog conjunctivitis1Enterobacter amnigenus biotype II 96%Enterobacter amnigenus biotype II 96%	37 Dog conju	nctivitis	1	Enterobacter amnigenus biotype II 96%	Enterobacter amnigenus biotype II 96%
38 Chicken samples, animal feed, cow milk, dog otitis8Enterobacter cloacae 94.7%Enterobacter cloacae 95.2%	38 Chicken sa feed, cow	mples, animal milk, dog otitis	8	Enterobacter cloacae 94.7%	Enterobacter cloacae 95.2%
39 Chicken bone marrow (2)3Escherichia coli 90.6%Escherichia coli 99.9%and yolk sac	39 Chicken bo and yolk sa	one marrow (2)	3	Escherichia coli 90.6%	Escherichia coli 99.9%
40 Chicken egg1Escherichia hermannii 96%Escherichia hermannii 97.7%	40 Chicken eg	g	1	Escherichia hermannii 96%	Escherichia hermannii 97.7%
41 Canary leg necrosis, human urine2Klebsiella pneumoniae subsp. pneumoniae 94.5%Klebsiella pneumoniae subsp. pneumoniae 98.2%	41 Canary leg human urir	necrosis, ne	2	Klebsiella pneumoniae subsp. pneumoniae 94.5%	Klebsiella pneumoniae subsp. pneumoniae 98.2%
42 Guinea pig   1   Morganella morganii 94%   Morganella morganii 99.9%	42 Guinea pig		1	Morganella morganii 94%	Morganella morganii 99.9%
43 Pork meat, animal feed, 3 Proteus mirabilis 92% Proteus mirabilis 99.9% chicken feces	43 Pork meat, chicken fee	animal feed,	3	Proteus mirabilis 92%	Proteus mirabilis 99.9%
44 Chicken feces1Providencia rettgeri 92%Providencia rettgeri (no id%)	44 Chicken fe	ces	1	Providencia rettgeri 92%	Providencia rettgeri (no id%)
45 Chicken feces 1 Rahnella aquatilis 95% Rahnella aquatilis	45 Chicken fe	ces	1	Rahnella aquatilis 95%	Rahnella aquatilis

				(no id%)
46 1	Human skin, shampoo sample	2	Serratia marcescens 88.5%	<i>Serratia marcescens</i> 97.4% (no id% for 1 strain)
47 (	Cow milk	1	Serratia liquefaciens 82%	Serratia liquefaciens 99.8%
48 ( 1 1 1 5	Chicken liver and feces, pigeon samples, guinea pig bone marrow, animal feed, wild boar liver, pig samples	21	Salmonella spp.91.3%	Salmonella spp.91.1% (no id% for 8 strains)
49 (	Chicken feces	1	Shigella sonnei 94%	Shigella sonnei 48.6%

Legend: a- ABIS %id represents the percentage of similarity with taxa from the database, all databases containing a matrix where probabilistic incidence values are allocated for every taxon and their corresponding morpho-biochemical characters; b- apiweb<sup>TM</sup> %id is a probabilistic calculation using bioMerieux own system procedure.

Apiweb<sup>TM</sup> returned an id% lower than 80% for 9 species (*Staphylococcus schleiferi*, *S. haemolyticus*, *Stenotrophomonas maltophilia*, *Salmonella spp.*, *Shigella sonnei*, *Streptococcus pyogenes*, *Streptococcus bovis*, *Mannheimia haemolytica*, and *Lactobacillus fermentum*), while ABIS returned low id% for 2 species (*Bacillus pumilus* and *Vibrio parahaemolyticus*).

For the reference strain *Clostridium tetani* RO-2009 apiweb<sup>TM</sup> returned *Clostridium tetani* without calculating an id%, as an alternative result after *Egerthella lenta* 75%, *Clostridium spp.* 16.9% and *Clostridium hystolyticum* 3%, while ABIS result was *Clostridium tetani* 97%.

Proteus hauseri is biochemically similar to strains commonly identified as Proteus vulgaris, but esculin and salicin negative. Since these tests were not available neither in lab, nor in API galleries, the ATCC 13315 - Proteus hauseri strain was identified by ABIS as "P. vulgaris / P. hauseri", and by apiweb<sup>TM</sup> as "P. vulgaris group".

When identifying Salmonella, results were almost similar, the id% differing only by 0.2%. Moreover, 17 Salmonella strains were serologically confirmed by "O"antisera agglutination, 1 strain was confirmed by a national reference lab as Salmonella Typhimurium, and 1 strain was confirmed by a national reference lab as Salmonella Kottbus.

When identifying *Mannheimia haemolytica*, apiweb<sup>TM</sup> could not differentiate *M. haemolytica* from *Pasteurella trehalosi*. In one case ABIS returned *Mannheimia* 

*glucosida* as an alternative result. The validated result was *M. haemolytica* regarding ecology and pathogenicity.

When identifying *Streptococcus pyogenes*, for one strain apiweb<sup>TM</sup> returned an alternative option: *Streptococcus oralis*, while ABIS returned *Gemella palaticanis* as an alternative. In both cases the final result was *S. pyogenes* regarding strain's ecology and pathogenicity.

When identifying *Staphylococcus xylosus*, ABIS returned multiple choices including: *S. gallinarum*, *S. nepalensis* and *S. equorum*. The validation of *S. xylosus* as final result is doubtful since apiweb<sup>TM</sup> database does not include the above taxa.

Comparison of the average id% of wild strains per ABIS and apiweb<sup>™</sup> databases shows that ABIS got 13.6% higher identification score for staphylococci (93.5% vs 79.9%), 2.2% higher for streptococci (90.7% vs 88.5%), 1.1% lower for *Enterobacteriaceae* (92.9% vs 94.0%) and 2.9% lower for other bacteria (87.8% vs 90.7%). ABIS's average id% of reference strains was 5.0% lower than apiweb's (93.9% vs 98.9%) (fig. 4).

Overall, ABIS achieved an average id% of 91.8% and apiweb<sup>TM</sup> 90.4%, ABIS showing an identification percent higher by 1.4%. Moreover, ABIS results have been correlated with ecological, morphological, biochemical and pathological characters of the identified taxa.

The variation range of apiweb<sup>TM</sup> id% (43.9 to 99%) was wider than ABIS (77 to 99%) which shows that the ABIS system is more homogenous and consistent.





## Conclusion

Apiweb<sup>™</sup> confirmed the results of ABIS with a high id%, proving very good taxa identification by ABIS program. ABIS online (*ABIS online - bacterial identification software* version 12, http://www.tgw1916.net) is capable of correct phenotypical species identification, generates reliable results, is a powerful tool for microbiology lab and the Encyclopedia connection provides essential information about the ecological significance, pathology and other features of the identified strains.

## References

- Borriello SP, Murray PR, Funke G (eds.). Topley & Wilson's Microbiology & Microbial Infections, Bacteriology, vol 2. Edward Arnold (Publishers) Ltd, London, 10th ed., 2006.
- 2. Buiuc D, Neguț M (eds). Tratat de microbiologie clinică. Editura Medicală, București, ediția a III-a, 2009.
- Gordon RE, Haynes WC, Pang CHN. The Genus Bacillus, Agriculture Handbook No.427. USDA, Washington DC, 1973.

- Dworkin M, Falkow S, Rosenberg E, Schleifer KH (eds). The Prokaryotes, a handbook on the biology of bacteria, Vol. 4, Bacteria: Firmicutes, Cyanobacteria. Springer, New York, 3rd ed, 2006.
- Buchanan RE, Gibbons NE (eds). Bergey's Manual of Determinative Bacteriology. Williams & Wilkins, A Waverly Company, Baltimore, 8th ed., 1975.
- Holt JG, Krieg NR, Sneath PHA, Staley JT et al. (eds). Bergey's Manual of Determinative Bacteriology. Williams & Wilkins, A Waverly Company, Baltimore, 9th ed., 1994.
- Vos PD, Garrity G, Jones D, Krieg NR et al. (eds). Bergey's Manual of Systematic Bacteriology, Vol. 3: The Firmicutes. Springer, New York, 2nd ed, 2009.
- Goodfellow M, Kampfer P, Busse HJ, Trujillo et al. (eds). Bergey's Manual of Systematic Bacteriology, Vol. 5: The Actinobacteria, Part A. Springer, New York, 2nd ed., 2012.
- 9. Garrity GM (ed). Bergey's Manual of Systematic Bacteriology, Vol. 2: The Proteobacteria , Part B. Springer, New York, 2nd ed, 2004.
- Funke G, von Graevenitz A, Clarridge III JE, Bernard KA. Clinical Microbiology of Coryneform Bacteria. *Clin Microbiol Rev.* 1997;10(1): 125-159. doi: 10.1128/CMR.10.1.12