Drug resistance in *Trueperella pyogenes* human and animal clinical isolates in Switzerland

Emma Marchionatti[1,2], Sonja Kittl[2], Parham Sendi[3], Vincent Perreten[2]

[1] Farm Animal Clinic, Department of Clinical Veterinary Science, Vetsuisse Faculty, University of Bern
[2] Institute of Veterinary Bacteriology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern
[3] Institute for Infectious Diseases, Faculty of Medicine, University of Bern

INTRODUCTION

Trueperella pyogenes is a Gram-positive, non-spore-forming, non-motile, non-capsulated, facultative-anaerobic rod, belonging to the skin and mucous membranes of the upper respiratory, urogenital and gastrointestinal tracts biota of animals. *T. pyogenes* is also an opportunistic pathogen causing suppurative infections in animals, mostly farm animals, and rarely humans (1). These infections are commonly treated with antibiotics, posing the risk of selecting resistant bacteria through the acquisition of mobile genetic elements containing antimicrobial resistance genes (2). The objectives of the present study were i) to describe the phenotypic and genotypic antimicrobial resistance profiles of *T. pyogenes* in Switzerland, ii) to determine the genetic relatedness between strains of different origins. This study will also serve as a baseline for further investigations of mobile genetic elements and molecular epidemiology.

METHODS

Selection of *Trueperella pyogenes* strains

Thirty-one animal and 8 human *T. pyogenes* strains were selected from the cryopreserved collections of the Institute of Veterinary Bacteriology and of the Institute for Infectious Diseases, University of Bern, Switzerland. Four additional strains from cattle, previously sequenced and deposited in the NCBI database (CP081508, CP096279, CP093279, CP097247), were added to the study population (3). The strains were isolated on TSA-S (Becton, Dickson) after 5% CO_2 incubation at 37°C for 24 hours. Identification was confirmed by MALDI-TOF mass spectrometry (Bruker).

Antimicrobial susceptibility tests

Minimal inhibitory concentration (MIC) of 19 antibiotics and 3 antiseptics was determined by broth microdilution method using Sensititre EUST2 plates (Thermo Fisher) and home-made microtiter plates, respectively, and following CLSI recommendations. As no *T. pyogenes*' specific breakpoints are currently available, the MICs were interpreted using breakpoints derived from previously published data.

Whole genome sequencing (WGS) and WGS-based genetic characterization

Genomic DNA was extracted using Masterpure™ purification kit (Lucigen) and sequenced at the NGS Platform, University of Bern, Switzerland, using PacBio HiFi technology. PacBio HiFi reads were demultiplexed and de novo assembled using Flye v.2.9.1. The 43 complete genomes were screened *in silico* for antimicrobial resistance genes with Resfinder v.4.1 (Center for Genomic Epidemiology) and CARD-RGI (McMaster University). A whole genome-based taxonomic analysis was performed using Type (Strain) Genome Server (Leibniz Institute DSMZ) for phylogenetic relatedness.

Table 1. Minimal inhibitory concentration of 22 antimicrobial agents for 43 *T. pyogenes* clinical isolates in Switzerland

	Number of isolates classified by their MIC values (µg/mL)																			
Antimicrobial	**≤**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>**	**MIC_{50}**	**MIC_{90}**
Kanamycin									43	|									≤4	≤4
Rifampicin	46									╟									≤0.015	≤0.015
Erythromycin					38		2	╟┤	╟	1	1	1							≤0.25	0.5
Ciprofloxacin							1	31	9	2		╟┤							1	2
Tetracycline						20		╫		╟	7	6╟	10						8	>16
Streptomycin									23	|	1	6	6	7				<4	>32
Tiamulin						43						╫							≤0.5	≤0.5
Quinupristin/dalfopristin						43													≤0.5	≤0.5
Linezolid							43												≤1	≤1
Mupirocin																	43		>256	>256
Vancomycin							43												≤1	≤1
Clindamycin					39				╫	╟	3								≤0.12	≤0.12
Cefoxitin						42					1								≤0.5	1
Gentamicin						31		11	╟┤	╫		|	1						1	2
Sulfamethoxazole													26			╫		17╟	≤64	>512
Chloramphenicol									42		1╟								≤4	≤4
Fusidate					43														≤0.25	≤0.25
Penicillin				42╟		1╫			┤	╟									≤0.06	≤0.06
Trimethoprim							40					1	2						≤1	≤1
Chlorhexidine digluconate									12	31									4	4
Octenidine dihydrochloride					31	12													≤0.25	0.25
Alkylbenzyldimethylammonium chloride								7	35	1									2	2

White area: dilution ranges in µg/mL of each antimicrobial tested; Gray area: number of isolates with a MIC ≤ or > the lowest/highest concentration tested; MIC breakpoints from | (4), ╟ (5), ┤ (6), ╫ (7), ╟ (8)

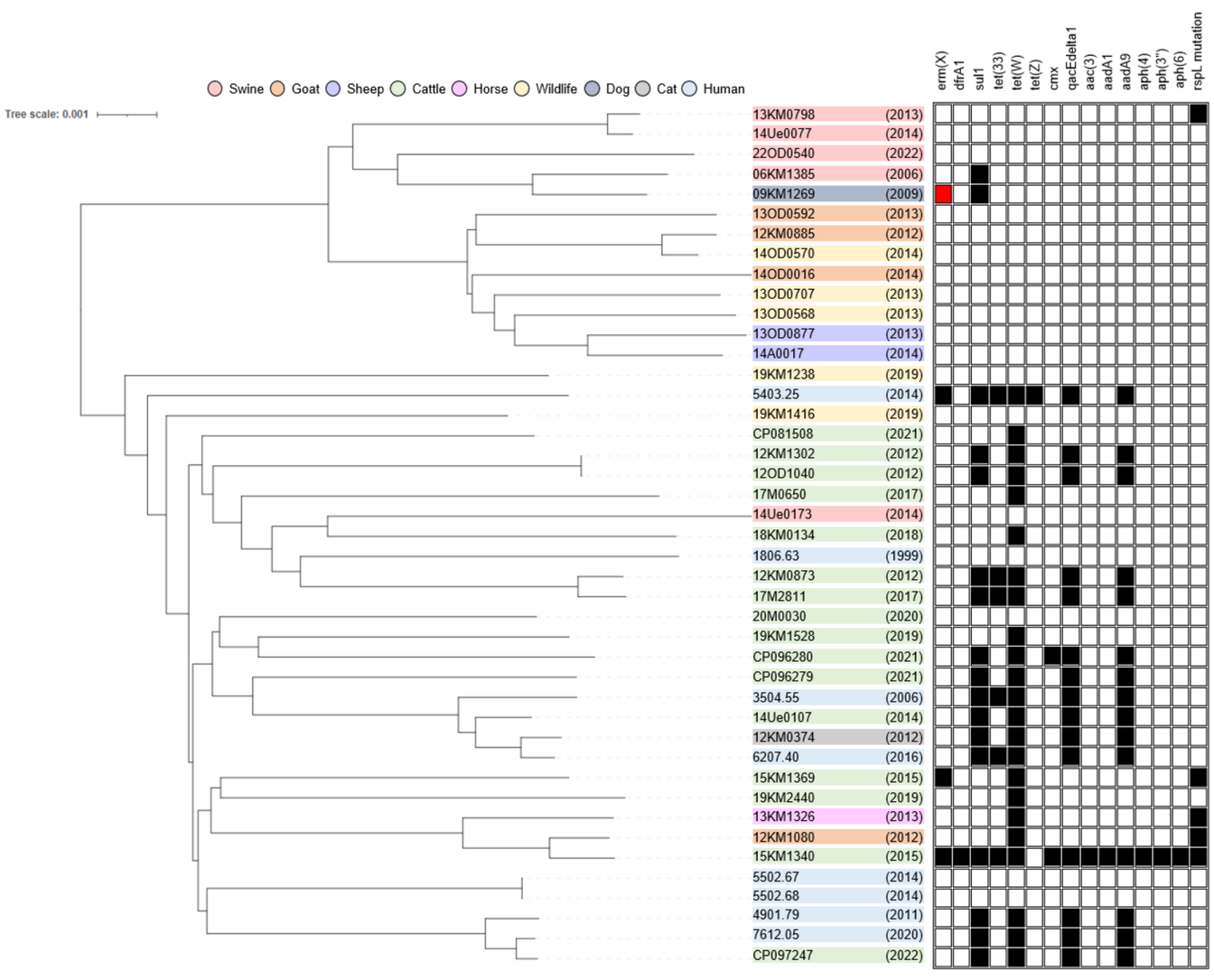

Figure 1. Core genome phylogeny and *in silico* antibiograms of 43 T. pyogenes genomes isolated in Switzerland. The genomic features for antimicrobial resistance are represented by their presence (black square) or abscence (white square); red square represents a new erm gene.

RESULTS and DISCUSSION

MIC distribution of the 22 antimicrobial agents tested for the 43 *T. pyogenes* clinical isolates are reported in Table 1. *T. pyogenes* phenotypic resistance to tetracycline (n=23), aminoglycosides (n=20), and sulfonamides (n=17) is common, while resistance to macrolides (n=3), trimethoprim (n=2), and chloramphenicol (n=1) is rare in this study population.

The phylogenetic relationship between the 43 strains of *T. pyogenes*, as well as the presence of antimicrobial resistance genes, are reported in Figure 1. Genetic analysis revealed 2 large clusters, where human isolates are more closely related to isolates from cattle. Furthermore, phenotypic vs genotypic analysis permitted the detection of a putative new resistance mechanism (strain 09KM1269).

The presence of antimicrobial resistance genes in *T. pyogenes* indicates that judicious use of antibiotics should be made based on antimicrobial susceptibility testing.

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