

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

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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% CO₂ 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

Antimicrobial	Number of isolates classified by their MIC values (µg/mL)																	MIC ₅₀	MIC ₉₀	
	≤	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512			>
Kanamycin									43										≤4	≤4
Rifampicin	46																		≤0.015	≤0.015
Erythromycin					38		2				1		1						≤0.25	0.5
Ciprofloxacin							1	31	9		2								1	2
Tetracycline											7	6							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	2
Sulfamethoxazole																26			≤64	>512
Chloramphenicol																			≤4	≤4
Fusidate									43										≤0.25	≤0.25
Penicillin									42										≤0.06	≤0.06
Trimethoprim									40										≤1	≤1
Chlorhexidine digluconate																			4	4
Octenidine dihydrochloride									31		12								≤0.25	0.25
Alkylbenzyltrimethylammonium 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)

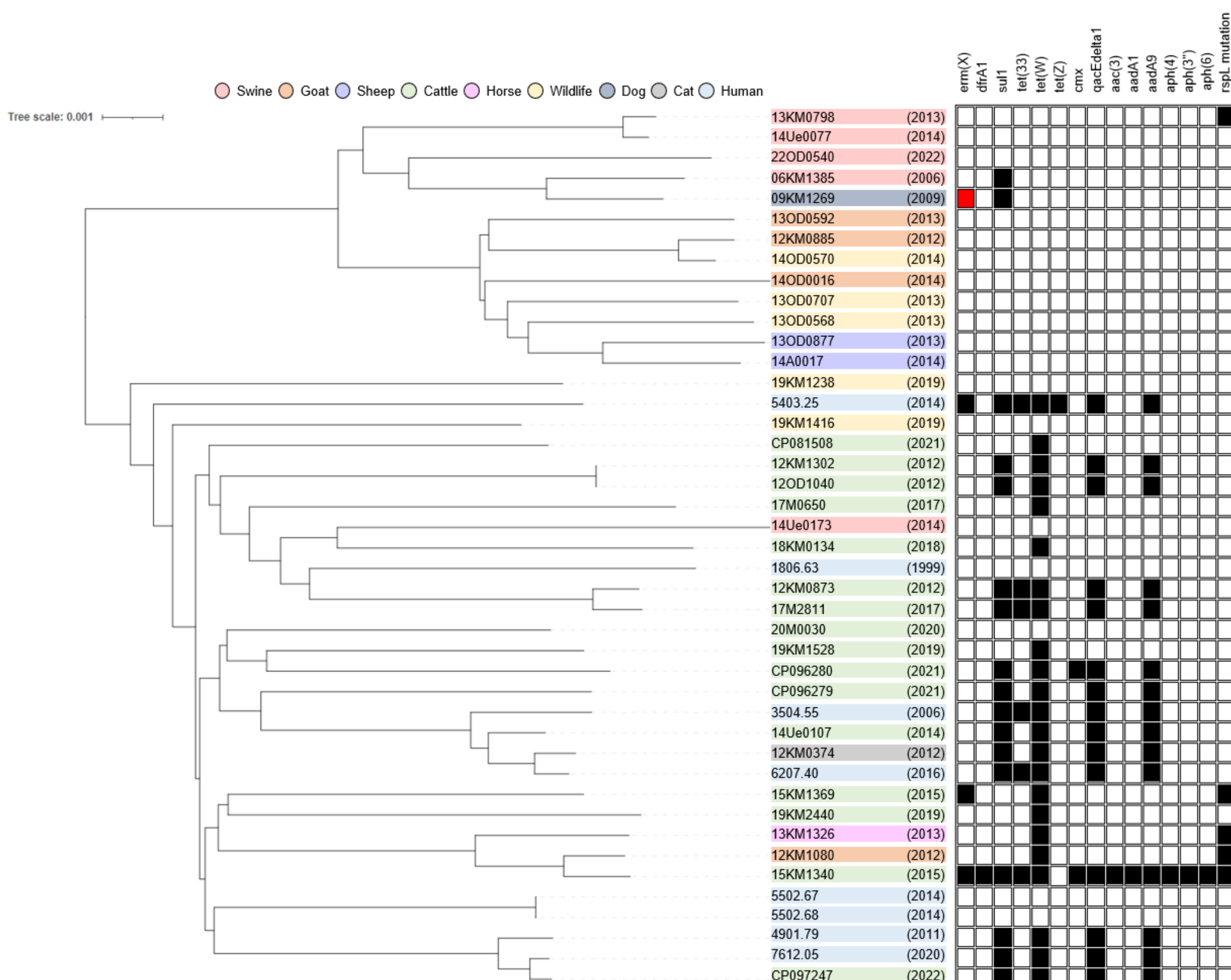


Figure 1. Core genome phylogeny and *in silico* antibiograms of 43 *T. pyogenes* genomes isolated in Switzerland. The genomic features for antimicrobial resistance are represented by their presence (black square) or absence (white square); red square represents a new erm gene.

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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