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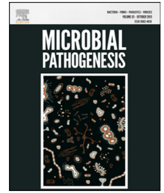

Relationships between antimicrobial resistance, distribution of virulence factor genes and the origin of *Trueperella pyogenes* isolated from domestic animals and European bison (*Bison bonasus*)

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ABSTRACT

Trueperella pyogenes is an opportunistic pathogen causing suppurative infections in livestock and wild animals. Although this bacterium is known for a long time, our knowledge about its pathogenicity is still insufficient. In this study the relationships between antimicrobial resistance profiles, distribution of virulence factor genes and the origin of *T. pyogenes* isolates were investigated. Isolates ($n = 97$) from various infections in domestic animals and European bison were studied. Minimal inhibitory concentrations of 12 antimicrobials were determined by a strip diffusion method, and PCR was used for detection of genes encoding seven putative virulence factors. All strains were susceptible to tested beta-lactams, and a statistically significant correlation between the resistance to enrofloxacin, tetracycline, macrolides, clindamycin, and a strain origin was found. The isolates from European bison were more susceptible than those from livestock, however the resistance to tetracycline and fluoroquinolones was observed. The *plo* and *fimA* genes were detected in all strains. There was no statistically significant association between the distribution of particular virulence factor genes and the type of infection, but the *nanH*, *nanP* and *fimG* genes were less frequently found in the isolates from European bison. The presence of three genes, *nanP*, *nanH* and *cbpA*, was found to be related to the resistance to tetracycline and ciprofloxacin. In conclusion, the resistance patterns of *T. pyogenes* were correlated with an isolate origin, but our findings did not allow to indicate which of the putative virulence factors may play a crucial role in the pathogenesis of particular types of *T. pyogenes* infection.

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1. Introduction

Trueperella pyogenes is a non-motile, non-spore-forming, pleomorphic bacterium, belonging to the class *Actinobacteria*. It is an inhabitant of mucus membranes of the upper respiratory, urogenital and gastrointestinal tracts of animals. *T. pyogenes* is pathogenic for different animal species, and purulent lesions may occur in various tissues. In domestic animals, mainly ruminants and swine, *T. pyogenes* causes various diseases, including metritis, mastitis, liver abscessation, pneumonia, arthritis, endocarditis and osteomyelitis [1–3]. In wild animals such as antelopes, camels, elephants, macaws, deer, bison (*Bison bison*), European bison (*Bison bonasus*), it is a cause of abscesses of various localization and urogenital tract infections [2,4–8]. In humans *T. pyogenes* infections are rare [9]. Although *T. pyogenes* is a bacterium known for a long time, its pathogenic properties, routes of infection and the relationship between strains infecting various animal species are still poorly understood. Therefore this study was focused on *T. pyogenes* properties associated with pathogenicity, such as antimicrobial susceptibility and the presence of genes encoding putative virulence factors in strains of various origin.

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The beta-lactam antibiotics, tetracyclines, macrolides and fluoroquinolones are antimicrobials commonly used to treat *T. pyogenes* infections in domestic animals as well as in captive wild animals [1,7,10]. In case of those infections an extensive antibiotic therapy is often used and in some countries antimicrobials are administrated in sub-therapeutic doses as prophylaxis against the infections in cattle [2,11]. However, this may result in an increasing resistance of the pathogen. The majority of available data concerns the resistance of isolates from cattle and swine [3,10,12–15]. There is little information on the resistance mechanisms in *T. pyogenes*. Various mobile genetic elements may play an essential role in the dissemination of antimicrobial resistance in *T. pyogenes*. It was found that integron-gene cassettes encoding aminoglycoside and beta-lactam resistance genes can be present in *T. pyogenes*, mainly in multidrug-resistant (MDR) strains [8,13]. The tetracycline resistance in *T. pyogenes* is associated with the presence of the *tet*(W) gene, located on three various transposons, which is widely disseminated among isolates of various origin [16]. The macrolide resistance in *T. pyogenes*, determined by the presence of the *erm*(X) and *erm*(B) genes, may have inducible or constitutive character [11,17].

The pathogenic potential of *T. pyogenes* is determined by several putative virulence factors. Pyolysin (PLO) is a major virulence factor, which is responsible for lysis of host cells, and the *plo* gene is found in all *T. pyogenes* strains [2]. A collagen-binding protein (CbpA), encoded by the *cbpA* gene, plays an important role in adhesion to host cells, especially in collagen-rich tissues [18]. Two neuraminidases, H (NanH) and P (NanP), also contribute to the colonization of host tissues, probably in the first stage of infection [2]. Other putative determinants of *T. pyogenes* virulence are fimbriae, which are probably involved in the adhesion to host cells [2]. The genes *fimA*, *fimC* and *fimG* encode subunits of type A fimbria (FimA), type C fimbria (FimC) and type G fimbria (FimG), respectively. The *cbpA*, *nanH*, *nanP* and fimbrial genes occur with variable frequency in *T. pyogenes* isolates. Other properties such as the ability to survive within host phagocytes and to biofilm formation may also be involved in *T. pyogenes* pathogenicity [2,19]. Most of published data concern *T. pyogenes* factors that may determine the development of metritis and mastitis in cattle [3,20,21]. However a role of particular bacterial factors in the pathogenesis of other types of *T. pyogenes* infection is poorly known.

The aim of this study was to determine the values of minimal inhibitory concentration (MIC) of selected antibiotics for *T. pyogenes* strains of various origin, and to investigate the relationships between antimicrobial resistance profiles, the distribution of virulence factor genes and an isolate origin.

2. Materials and methods

2.1. Bacterial strains

A total of 97 *T. pyogenes* strains isolated from various animal hosts in Poland were studied (31 from cattle, 26 from pigs, 9 from goats, 5 from sheep, 25 from European bison and 1 from antelope). The specimens were collected from animals with different types of infection. Studied *T. pyogenes* strains were obtained from following infections: mastitis (19 strains from cattle), metritis (10 strains from cattle), abscesses in various tissues (2 strains from cattle, 6 from swine, 9 from goats, 5 from sheep, 7 from European bison, 1 from antelope), pneumonia (20 strains from swine, 3 from European bison), and balanoposthitis (15 strains from European bison).

Samples from European bison were obtained during selective culling of free-living animals in the Białowieża Primeval Forest (the permission of the Office of Environment Protection in Warsaw nr WPN-I.6401.260.2015.EB.2), and one sample from antelope was recovered from a captive animal in the Warsaw Zoological Garden.

Bacteria were isolated on Columbia Agar supplemented with 5% sheep blood (bioMérieux, France) for 48 h at 37 °C in 5% CO_2. Isolates were identified based on their phenotypic properties as described previously [6].

The reference strain *T. pyogenes* ATCC® 19411 was included as a control for the screening of virulence factor genes by PCR. The reference strains, *Streptococcus pneumoniae* ATCC® 49619 and *Staphylococcus aureus* ATCC® 25923, were used for quality control of susceptibility testing according to the Clinical and Laboratory Standards Institute guidelines [22,23].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility of *T. pyogenes* was determined by the strip diffusion method using Etest® strips (bioMérieux, France). Twelve antimicrobials were tested: penicillin (PEN), amoxicillin/clavulanic acid (AMC), cephalothin (CEF), cefotaxime (CTX), ciprofloxacin (CIP), enrofloxacin (ENR), tetracycline (TET), erythromycin (ERY), azithromycin (AZM), clarithromycin (CLR), rifampicin (RIF) and clindamycin (CLI). The testing conditions used in the study and criteria of interpretation of the results were in accordance with the guidelines for *Corynebacterium* spp. and coryneforms, including the genera *Arcanobacterium* and *Trueperella*, in the Clinical and Laboratory Standards Institute document M45-Ed3 [22]. Mueller-Hinton agar supplemented with 5% sheep blood (Graso, Poland) was inoculated with bacterial suspension in saline (the density of 0.5 McFarland standard), Etest® strips were placed on the surface of the medium and the plates were incubated for 48 h at 37 °C in 5% CO_2. The MIC value was read at the point where the edge of the growth inhibition ellipse intersected the strip.

The MIC breakpoints applied in this study are shown in Table 1. The MIC results for penicillin, cefotaxime, ciprofloxacin, tetracycline, erythromycin, rifampicin and clindamycin were interpreted according to the criteria approved for coryneforms [22]. The MIC breakpoints approved for staphylococci in the CLSI document VET01S-Ed3 [23] were used for interpreting susceptibility to amoxicillin/clavulanic acid, cephalothin and enrofloxacin, and those approved for streptococci to interpret the results for azithromycin and clarithromycin.

2.3. Detection of genes encoding putative virulence factors

This part of the study was performed for all *T. pyogenes* isolates except those from European bison ($n = 25$) which had been investigated in our previous work [6].

The genomic DNA from 72 *T. pyogenes* strains was extracted using Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer's recommendations as described previously [6]. The purity and quantity of DNA were determined by electrophoresis in 1% agarose gel with a molecular mass marker (MassRuler™DNA Ladder Mix; Thermo-Scientific).

The presence of genes encoding seven putative virulence factors of *T. pyogenes* (*plo*, *nanH*, *nanP*, *cbpA*, *fimA*, *fimC* and *fimG)* was investigated by PCR technique using primers and reaction conditions described previously [6]. PCR products were visualized and analyzed using a VersaDoc Model 1000 Imaging System and Quantity One software (version 4.4.0) (BioRad, USA).

2.4. Statistical analysis

Data were presented as counts and percentages. Ninety five percent confidence intervals (95% CI) were calculated for a proportion of resistant strains using Wilson score method. Proportions of resistant strains were compared between domestic and wild

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