Antimicrobial susceptibility of *Prototheca zopfii* isolated from bovine mastitis

Władysław Wawron, Mariola Bochniarz, Tomasz Piech, Jerzy Wysocki¹, Marcin Kocik²

Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine, University of Life Sciences, 20-612 Lublin, Poland

¹Veterinary Surgery, 16-080 Tykocin, Poland

²Veterinary Surgery, 32-825 Borzecin, Poland

wladyslaw.wawron@up.lublin.pl

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Abstract

The aim of the study was to evaluate the susceptibility of algae of the genus *Prototheca* to antifungal and antibacterial antibiotics. The study involved 27 isolates of *Prototheca zopfii* obtained from milk of mastitis-affected cows kept in a detached cowshed in the North-Eastern part of Poland. Analysis of *P. zopfii* susceptibility has demonstrated low effectiveness of both antifungal and antibacterial antibiotics. All algae isolated from milk were resistant to clotrimazole, fluconazole, econazole, flucytosine, cefoperazone, cephalixin, enrofloxacin, lincomycin, and oxytetracycline (100% of resistant isolates), as well as miconazole (92.6% of resistant isolates). Nystatin, ketoconazole, and amphotericin B showed the highest activity amongst the antifungal antibiotics (88.9% and 0.0%, 51.9% and 22.2%, 0.0% and 48.1% of susceptible and intermediate susceptible isolates, respectively). In the group of antibacterial antibiotics, the high activity against *P. zopfii* was observed only in the case of gentamicin, kanamycin (96.3% and 92.6% of susceptible isolates, respectively), and polymyxin B (59.3% of susceptible and 33.3% of intermediate susceptible isolates).

Key words: cows, mastitis, *Prototheca*, antibiotic susceptibility.

Introduction

Algae are ubiquitous, unicellular, achlorophyllic organisms existing in the natural environment (19). They belong to the genus *Prototheca*, the family *Chlorellaceae*. They are one of few plant-like organisms, which can cause infections in humans and animals (11-13, 18, 21). The species isolated from affected humans and animals include *P. zopfii*, *P. wickerhamii*, and *P. blaschkeae* (12, 20, 22, 23, 25, 27). The remaining species were considered as non-pathogenic.

Mastitis in dairy cows is one of the most important infections caused by *Prototheca* pathogens (17). The disease results in substantially decreased milk production and increased somatic cell count (8). It even can lead to cow culling, causing high financial losses.

Protothecosis of the udder is a serious therapeutic problem. According to the literature data, *Prototheca* sp. does not respond to the routine therapy of mastitis (9, 15, 16, 21). The studies concerning *in vitro* and *in vivo* susceptibility of algae indicate their high resistance to antibacterial and antifungal antibiotics (4-6, 11).

The algae in natural environment occur in places characterised by high humidity and high organic matter content (bedding, wastes, and animal faeces) (6, 16, 28). Therefore, the development of bovine udder infections induced by *Prototheca* pathogens is considered to be highly dependent on predisposing factors, i.e. unsuitable upkeep conditions and inappropriate hygiene of milking (8, 9, 11, 12, 21, 23). Spontaneous recoveries have not been noted.

The objective of the study was to evaluate antifungal and antibacterial antibiotic susceptibility of *Prototheca* algae isolated from mastitis-affected dairy cows.

Material and Methods

The study involved 27 *Prototheca zopfii* species isolated from milk of mastitis-affected cows. The study was carried out in the summer 2011. The cows...
originated from one herd in the North-Eastern part of Poland. The animals were kept in a detached cowshed.

The affected cows did not respond to routine antibiotic treatment. Milk was sampled according to the accepted procedure. Once the udder skin was cleansed, washed, and dried, and the teats disinfected with 70% alcohol, milk was collected to sterile, labelled test tubes without preservatives, chilled to 4°C and delivered to the laboratory for further examinations.

Bacteriological and mycological testing was carried out according to standard procedures: milk culture, using the agar medium plus 5% mutton blood, and Sabouraud agar with the addition of chloramphenicol, followed by 24-72 h incubation of plates under aerobic conditions at 37°C, and evaluation of morphology of colonies of isolated pathogens and Gram stained specimens. The isolated Prototheca sp. were identified using API 20C AUX (Biomerieux, France).

Antibiotic susceptibility of the pathogens was analysed using the disc diffusion method on the Mueller-Hilton agar according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

The testing of Prototheca zopfii susceptibility was performed with the following drugs: antimicotic - amphotericin B (20 μg), econazole (10 μg), fluconazole (10 μg), clotrimazole (10 μg), miconazole (10 μg), nystatin (100 U), and antibacterial - amoxicillin with clavulanic acid, enrofloxacin, cephalexin, cefoperazone, lincomycin, and oxytetracycline. The susceptibility was determined by measuring the zone of growth inhibition around the antibiotic-soaked disc (Oxoid, England). According to the size of inhibition zone, the strains were divided into 3 categories: susceptible (+), intermediately susceptible (+/-), and resistant (−).

Table 1. Susceptibility of Prototheca zopfii to antimicotic drugs

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<tr>
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<th>AMF</th>
<th>CLOT</th>
<th>ECO</th>
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<tr>
<td>R</td>
<td>14</td>
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<td>27</td>
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<td>27</td>
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</table>

S—susceptible; I—intermediately susceptible; R—resistant
AMF—amphotericin B; CLOT—clotrimazole; ECO—econazole; FLC—fluconazole; FLU—flucytosine; KCA—ketoconazole; MIC—miconazole; NYST—nystatin

Results

P. zopfii strains showed substantial in vitro resistance to antibiotics (Tables 1 and 2). All algae isolated from milk were unsusceptible to clotrimazole, fluconazole, econazole, and flucytosine (100.0% of resistant strains) (Table 1). Moreover, miconazole was also found to be ineffective (92.6% of resistant strains). The highest activity was demonstrated by nystatin (88.9% of susceptible strains), ketoconazole (51.9% of susceptible and 22.2% of intermediate susceptible strains), and amphotericin B (48.1% of intermediate susceptible strains).

The highest effectiveness amongst antibacterial antibiotics was demonstrated by gentamicin, kanamycin, and polymyxin B (Table 2). The percentages of strains susceptible and intermediately susceptible to these antibiotics were as follows: 96.3% and 3.7%, 92.6% and 3.7%, 59.3% and 33.3%, respectively. The remaining antibiotics, i.e. amoxicillin with clavulanic acid, enrofloxacin, cephalexin, cefoperazone, lincomycin, and oxytetracycline, were found to be ineffective in vitro (100.0% of resistant strains). The percentage of strains intermediately susceptible to colistin was high (29.6%).

Discussion

The analysis of antibiotic susceptibility of P. zopfii strains confirmed low effectiveness of antimicotic and antibacterial drugs. All algae isolated from milk were unsusceptible to clotrimazole, fluconazole, econazole, flucytosine, cefoperazone, cephalexin, enrofloxacin, lincomycin, and oxytetracycline (100.0% of resistant strains) as well as to miconazole and colistin (92.6.0% and 63.0% of resistant strains, respectively). Similarly, the combination of amoxicillin with clavulanic acid was found ineffective (100.0% of resistant strains). The results presented by Milanov et al. (17) were comparable; all algae isolated from milk were also unsusceptible to enrofloxacin, amoxicillin, tetracycline, penicillin, lincomycin, and novobiocin.
Table 2. Susceptibility of *Prototheca zopfii* to antibacterial drugs

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<tr>
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<th>AMC</th>
<th>CFP</th>
<th>CEF</th>
<th>COL</th>
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<td>100.0</td>
<td>17</td>
<td>63.0</td>
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</table>

S—susceptible; I—intermediately susceptible; R—resistant
AMC—amoxicillin with clavulanic acid; CFP—cefoperazone; CEF—cephalexin; COL—colistin; ENR—enrofloxacin; G—gentamicin; KAN—kanamycin; LINCO—lincomycin; T—oxytetracycline; POLI B—polymyxin B

The obtained findings revealed that the highest antimicrobial activity was demonstrated by nystatin, ketoconazole, and amphotericin B, which is consistent with the results of other authors confirming a high effectiveness of nystatin and amphotericin B against algae isolated from milk of mastitis-affected cows (5, 7, 14, 17). Moreover, the results reported by Malinowski et al. (10) were similar. In their study, the highest percentage of *Prototheca* species isolated from cow’s milk was susceptible to amphotericin B (35.7% of susceptible and 7.1% of intermediate susceptible strains) and nystatin (21.4% of susceptible and 64.3% of intermediate susceptible strains). The growth of some species was also inhibited by pimaricin (17.9% of susceptible strains). The strains susceptible to streptomycin, gentamicin, and polymyxin B were also isolated by Bodenhoff and Madsen (3). According to Milanov et al. (17), algae were immediately susceptible to gentamicin and neomycin; no strains susceptible to kanamycin were found.

The results of the study and previously published data (9, 15, 17) have revealed high resistance of *P. zopfii* to antibiotics which affects the efficacy of mastitis treatment.

References


