Antimicrobial activity of wild mushrooms extracts against clinical isolates resistant to different antibiotics

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Running Head: Antimicrobial activity of wild mushrooms

A**bstract**

Aim: This work aimed to screen the antimicrobial activity of aqueous methanolic extracts of 13 mushroom species, collected in Bragança, against several clinical isolates obtained in Hospital Center of Trás-os-Montes and Alto Douro, Portugal.

Methods and Results: Microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). MIC results showed that *Russula delica* and *Fistulina hepatica* extracts inhibited the growth of Gram negative (*Escherichia coli*, *Morganella morganni* and *Pasteurella multocida*) and Gram positive (*Staphylococcus aureus*, MRSA, *Enterococcus faecalis*, *Listeria monocytogenes*, *Streptococcus agalactiae* and *Streptococcus pyogenes*) bacteria. A bactericide effect of both extracts was observed in *P. multocida, S. agalactiae and S. pyogenes* with MBC of 20 mg/ml, 10 mg/ml and 5 mg/ml, respectively. *Lepista nuda* extract exhibited a bactericide effect upon *P. multocida* at 5 mg/ml, and inhibited *Proteus mirabilis* at 20 mg/ml. *Ramaria botrytis* extract showed activity against *E. faecalis* and *L. monocytogenes*, being bactericide for *P. multocida*, *S. agalactiae* (MBCs 20 mg/ml) and *S. pyogenes* (MBC 10 mg/ml). *Leucopaxillus giganteus* extract inhibited the growth of *E. coli* and *P. mirabilis*, being bactericide for *P. multocida*, *S. pyogenes* and *S. agalactiae*.

Conclusions: *Fistulina hepatica*, *Ramaria botrytis* and *Russula delica* are the most promising species as antimicrobial agents.

Significance and Impact of the Study: Mushrooms extracts could be an alternative as antimicrobials against pathogenic microorganisms resistant to conventional treatments.

Keywords: Wild mushrooms; Northeast Portugal; Antimicrobial activity; Clinical isolates

Introduction

The prevalence of infectious diseases is becoming a worldwide problem; antimicrobial drugs have long been used for prophylactic and therapeutic purposes, but the drug-resistant bacterial strains have creating serious treatment problems (Klein *et al.* 2007; Steinkraus *et al.* 2007). Several studies carried out in Portugal with microorganisms such as MRSA (meticiline-resistant *Staphylococcus aureus*) (EARSS 2007), *Enterococcus faecalis* (Novais *et al.* 2004), *Enterobacteriaceae* (Machado *et al.* 2007) and *Pseudomonas aeruginosa* (Cardoso *et al.* 2002), revealed their high susceptibility to antimicrobial agents and, moreover, their increasing global virulence. In particular, the incidence of MRSA, in Portuguese hospitals is one of the most important in Europe (45%) (EARSS 2007).

Recently, Trindade *et al.* (2009) reported that bacteria develop resistance to antibiotics through genome mutations that are crucial for their survival. Therefore, despite the impossibility to avoid bacteria evolution, it is important to choose the most adequate antibiotics to control such evolution in favor of human host.

The huge increase of resistances associated to diseases and mortality (Shorr *et al.* 2006) is exerting a considerable pressure in health systems, mainly at hospital level. Besides the multi-resistance problem, the nosocomial infections (health-care associated infections) are associated to high mortality, as also to the increase in the internment period and related costs (Orsi *et al.* 2002; Masterton *et al.* 2003; Wisplinghoff *et al.* 2004; Sŭljagić *et al.* 2005; Cosgrove 2006). Disease Control and Prevention (CDC) centers estimate that nosocomial infections are at least ca. 5% in all hospitalized patients, dealing to more than 2 millions of infections and 99.000 deaths *per* year (Wenzel and Edmond 2001; CDC 2003; CDC 2007).

This situation has forced the research of new antimicrobial substances effective against pathogenic microorganisms resistant to conventional treatments. Natural resources have been exploited in the last years and among them mushrooms could be an alternative as source of new antimicrobials.

The Northeast of Portugal is one of the European regions with higher biodiversity in wild mushrooms, most of them with a great gastronomic importance. Mushrooms have been recognized as functional foods and as a source for the development of medicines and nutraceuticals (Lindequist *et al.* 2005; Poucheret *et al.* 2006). They could also be a source of natural antibiotics. Some mushroom extracts, including *Laetiporus sulphureus* (Turkoglu *et al.* 2007), *Ganoderma lucidum* (Gao *et al.* 2005) and *Lentinus edodes* (Hatvani 2001) have already demonstrated antibacterial activity. Furthermore, some Portuguese species studied by our research group proved to be active against *Bacillus cereus, B. subtilis* and *S. aureus* (Barros *et al.* 2007b; Barros *et al.* 2008). Nevertheless, the reports available on literature are not related with multi-resistant bacteria (MRSA*, E. faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, P. aeruginosa)* that are a problem at hospital level due to the scarce options of active antibiotics.

Herein, the *in vitro* antimicrobial activity of 13 wild mushrooms aqueous methanolic extracts was screened against the above mentioned multi-resistant bacteria and other clinical isolates from CHTMAD – Hospital Center of Trás-os-Montes and Alto Douro, Portugal.

Materials and methods

Mushroom species

Thirteen mushroom species were collected in different ecosystems of the Trás-os-Montes region in the Northeast of Portugal in 2005-2010 Autumns (**Table 1)**. The morphological identification of the wild macrofungi was made according to macro and microscopic characteristics, and following several authors (Marchand 1971; Moser 1983; Bon 1988; Courtecuisse and Duhem 2005) and online keys (http://www.mycokey.com/). Representative voucher specimens were deposited at the herbarium of *Escola Superior Agrária* of *Instituto Politécnico de Bragança*. After taxonomic identification, the mushrooms were immediately lyophilized (Ly-8-FM-ULE, Snijders, Netherlands) and kept in the dark in hermetically sealed plastic bags up to the point of analysis.

Standards and Reagents

Methanol was of analytical grade purity from Lab-Scan (Lisbon, Portugal). The culture media Muller Hinton broth (MHB), Wilkins-Chalgren Broth (WCB) and Columbia agar (CA) with 5% horse blood were obtained from Biomerieux (Marcy l´ Etoile, France), respectively. The dye *p*-iodonitrotetrazolium chloride (INT) was purchased from Sigma–Aldrich (Spruce Street; St. Louis, USA) to be used as microbial growth indicator. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA) before use.

Extracts preparation

Each mushroom lyophilized sample (ca. 3 g) was extracted using a methanol:water (80:20; 30 ml) mixture at -20 °C for 6 h. After 15 min in an ultrasonic bath, the extract was centrifuged at 4000 *g* for 10 min and filtered through Whatman nº 4 paper. The residue was then extracted with two additional 30 ml portions of the methanol:water

mixture. The combined extracts were evaporated at 40 ºC under reduced pressure to remove methanol (rotary evaporator Büchi R-210, Flawil, Switzerland), lyophilized, redissolved in water, at a concentration of 200 mg/ml, and stored at -20 ºC for further use.

Microorganisms and culture media

The microorganisms used were clinical isolates from patients hospitalized in various departments of the Hospital Center of Trás-os-Montes and Alto Douro – Chaves, Portugal.

Seven Gram negative bacteria (*Escherichia coli, Klebsiella pneumonia*, *Proteus mirabilis*, *Morganella morganii*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii, i*solated from urine and *Pasteurella multocida* isolated from synozial fluid) and nine Gram positive bacteria (*Staphylococcus aureus* and MRSA *i*solated from wound exudates, *Staphylococcus epidermidis*, *Staphylococcus hominis, Staphylococcus saprophyticus, Enterococcus faecalis* and *Listeria monocytogenes* isolated from blood culture*, Streptococcus agalactiae i*solated from vaginal swab and *Streptococcus pyogenes* isolated from oropharyngeal swab) were used to screen the antimicrobial activity of the mushroom extracts. All strains were identified using the MicroScan® panels automated methodology - Siemens.

Muller Hinton broth (MHB) and Wilkins-Chalgren Broth (WCB) were used for determination of Minimum Inhibitory Concentration (MIC, lowest concentration of the mushroom extract able to inhibit bacterial growth), while Columbia agar with 5% horse blood (CA) was used for determination of Minimum Bactericidal Concentration (MBC, the lowest concentration of mushroom extract at which bacterial growth was prevented, and the initial viability was reduced by at least 99.9 %).

Characterization of antibiotic susceptibility of target strains

The characterization of antibiotic susceptibility and the identification of the target strains were performed using MicroScan® panels (Siemens). These panels allow the simultaneous determination of susceptibility to antimicrobial agents and the strain identification, including aerobic and facultative anaerobic Gram negative bacilli (MicroScan® Neg panels), and Gram positive cocci such as some fastidious aerobic Gram positive and *Listeria monocytogenes* (MicroScan® Pos panels).

Antimicrobial susceptibility test entail miniaturizations of the dilution susceptibility test, where each antibiotic has been dehydrated. Each antimicrobial agent was previously diluted in Muller-Hinton broth with additional supplementation to concentrations with clinical interest. Breakpoint panels used concentrations equivalent to the standard breakpoints of CLSI (Clinical and Laboratory Standards Institute). After inoculation and rehydration with a standardized microorganism suspension, and further incubation at 37º C for at least 16 h, MICs values for each antibiotic were determined (SHD 2008).

Determination of antimicrobial activity of extracts

MIC determinations were performed by the microdilution method and the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay following the methodology suggested by Kuete *et al.* (2011a, b) with some modifications.

Initially, 50 µl of each mushroom extract (200 mg/ml) was diluted in 450 µl of MHB for all microorganisms, except for *Streptococcus pyogenes,* where WCB was used (final concentration of 20 mg/ml) and then, 200 µl of this extract solution was added in each well (96-well microplate). Dilutions were carried out over the wells containing 100 µl

of MHB or WCB and afterwards, 10 µl of inoculum (1 $\times 10^8$ cfu/ml) were added to all the wells. Two negative (one with MHB or WCB and the other with the mushroom extract) and one positive (with MHB or WCB and the inoculum) controls were performed. The plates were incubated at 37 °C, for 24 h, in an oven (Jouan, Berlin, Germany) or with humidified atmosphere containing 10% CO_2 (NuAire, Plymouth, USA), in the case of *Streptococcus*.

The MIC of the samples were detected following addition of INT (0.2 mg/ml, 40 µl) and incubation at 37 °C for 30 min. Viable microorganisms reduced the yellow dye to a pink colour. MIC was defined as the lowest mushroom extract concentration that prevented this change and exhibited complete inhibition of bacterial growth. For the determination of MBC, a portion of liquid (50 µl) from each well without changes in colour was plated on CA and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC. All the assays were carried out in duplicate.

Results

Analysis of antibiotic susceptibility showed that some of the clinical isolates used in the present work are multi-resistant bacteria (**Tables 2** and **3**) being a problem at hospital level due to the scarce options of active antibiotics. *A. baumannii* and *M. morgani* are the Gram negative bacteria with the highest number of resistances, being evident their low sensibility to beta-lactamics group. *E. coli* shows some resistance to beta-lactamics antibiotics, but also to quinolones group. Furthermore, the latter group shows low activity against *P. mirabilis* (**Table 2**). All the *Staphylococcus* strains, unless *S. saprophyticus*, are beta-lactamases producers. Among them, only *S. aureus* presented

sensibility to oxacillin. The Streptococcus strains, *L. monocytogenes* and *P. multocida*, presented high sensibility to all the tested antibiotics (**Table 3**).

Therefore, it is important to find new antimicrobials effective against these pathogenic microorganisms resistant to conventional treatments. Herein, wild mushrooms were evaluated as an alternative. Data obtained in the screening of antimicrobial activity of the different wild mushrooms extracts against Gram negative bacteria are shown in **Table 4**. It should be pointed out that the majority of the extracts did not present antimicrobial activity against the tested Gram negative bacilli, which is in agreement with the results previously reported by our research group concerning *E. coli*, *K. pneumonia* and *P. aeruginosa* (Barros *et al.* 2008b; Cruz *et al.* 2008), even when higher extract concentrations were used (Barros *et al.* 2007b). Nevertheless, a recent study describes methanolic extracts obtained from *Agaricus bisporus* and *Cantharellus cibarius* (from black sea region of Turkey) as having high antibacterial activity against *E. coli*, assessed by disc diffusion method (Ozen *et al.* 2011). The extraction solvent or even the mushrooms origin, as well as the bacterial strain could explain the differences in the antimicrobial activity reported by different authors for the same species.

In the current study none of the tested extracts inhibited *A. baumannii*, *K. pneumonia* or *P. aeruginosa.* However, *Lepista nuda* extract showed some antimicrobial activity against *P. mirabilis* and *P. multocida* with MIC values of 20 mg/ml and 5 mg/ml, respectively. This extract was completely inactive for the other tested bacilli (*E. coli*, *K. pneumonia* and *P. aeruginosa*) which is in agreement with the reported by Barros *et al.* (2008b) for the same mushrooms species. *Russula delica* and *Fistulina hepatica* extracts exhibited antimicrobial activity against *E. coli*, *M. morganni* and *P. multocida* at the highest tested concentration (20 mg/ml). *Leucopaxillus giganteus* extract also revealed activity against *E. coli*, *P. mirabilis* (MICs 20 mg/ml) and *P. multocida* (MIC 10 mg/ml) (**Table 4**).

Among the tested Gram negative bacteria, *P. multocida* was the most susceptible to the extracts.

The results obtained in the screening of antimicrobial activity of the different wild mushrooms extracts against Gram positive bacteria are shown in **Table 5**. *Agaricus arvensis* extract showed the lowest antimicrobial activity, being only effective against *S. pyogenes* (MIC 10 mg/ml). As observed for Gram negative bacteria, *Russula delica* and *Fistulina hepatica* extracts showed the highest activity also against Gram positive bacteria (almost all the tested bacteria, except for *S. hominis* and *S. saprophyticus*, respectively) with MICs between 5 mg/ml and 20 mg/ml. Other studies reported the high susceptibility of Gram positive bacilli to mushrooms extracts (Barros *et al.* 2007b; Barros *et al.* 2008a; Barros *et al.* 2008b). *Russula delica*, *Fistulina hepatica* and *Ramaria botrytis* extracts were the only samples showing antimicrobial activity against *E. faecalis*. Furthermore, *Triholoma portentosum, Sarcodon imbricatus* and *Mycena rosea* presented MICs of 20 mg/dl for *S. aureus* and MRSA.

Cantharellus cibarius, *Lepista nuda* and *Ramaria botrytis* extracts did not show any activity against *S. aureus* and MRSA, at the tested concentrations (up to 20 mg/ml). Nevertheless, a previous report described high antimicrobial activity of those species against *S. aureus* isolated from pus (MIC 5 mg/ml) (Barros *et al.* 2008b). It should be highlighted that the authors used a different extraction solvent (methanol), a different antimicrobial activity assay (agar streak dilution method based on radial diffusion), they dissolved the extracts in DMSO and not in water as in the present study, and specially they used a different strain, probably with a different antibiotic resistance profile.

A bactericidal effect of some of the studied mushrooms extracts was observed for the streptococci and *P. multocida* (**Table 6**). The lowest MBC values (5 mg/ml) for *S.*

pyogenes, *S. agalactiae* and *P. multocida* were achieved by *Russula delica*/*Fistulina hepatica*, *Leucopaxillus giganteus* and *Lepista nuda* extracts, respectively.

Discussion

The obtained results seem to be promising since some extracts were bacteriostatic for Gram negative bacteria and bactericidal for some Gram positive bacteria. The susceptible bacteria included *M. morganni*, MRSA, *S. epidermidis* and *E. faecalis*, which are multi-resistant (**Table 2 and 3**) and responsible for nosocomial infections. It is estimated that this kind of infections affects 4.1 millions of patients in EU each year, being responsible for a considerable increase of several diseases, mortality and related costs (Puupponen-Pimia *et al.* 2001). Although this *S. agalactiae* possess reduced resistance, there are already some reports describing *S. agalactiae* resistant to penicillin, ampicillin, erythromycin and clindamycin in different countries (Public Health; Cowan 1999; Kuete 2010).

Phenolic compounds might be some of the individual molecules present in the extracts responsible for the bioactivity, since methanol:water was used as solvent extraction. In fact, several authors have already related the antimicrobial activity of different natural matrixes with the presence and content of phenolic compounds (McGavin *et al.* 2001; D´Oliveira *et al.* 2003; Barros *et al.* 2007b).

The best results were obtained with the most sensitive microorganisms such as *P. multocida*, *L. momocytogenes* and *Streptococcus* group. Nevertheless, it should be highlighted that the tested mushroom extracts revealed the same behavior against the methicillin susceptible *S. aureus* (a MSSA) and MRSA. Therefore, more studies with *S. aureus,* but with different resistance profiles, should be performed in order to evaluate their behavior in the presence of a specific extract.

Overall, antimicrobial drugs have long been used for prophylactic and therapeutic purposes; however the drug-resistant bacterial strains have been creating serious treatment problems. This situation has forced the search of new antimicrobial substances effective against pathogenic microorganisms resistant to conventional treatments. Natural resources, such as the herein studied wild mushrooms, could be an alternative being included in diet taking advantage on the synergistic effects of all the compounds present, or giving extracts and isolated compounds that could be used as nutraceuticals or drugs. The studied mushrooms are edible species, and therefore, their ingestion should not bring toxicity. However, the toxicity of their extracts and individual compounds should be evaluated for further conclusions. Moreover, the mechanism of bacteriostatic or bactericide action of the best extracts (e.g. *Fistulina hepatica*, *Ramaria botrytis* and *Russula delica*) should be elucidated and the individual/combined phenolic compounds found in those extracts might be tested against selected bacteria in order to identify molecules responsible for the mushrooms bioactivity.

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Table 1. Information on the wild mushroom samples collected.

Scientific name	English name	Ecology	Habitat
Agaricus arvensis (Shaeff.:Fr.)	Horse Mushroom	Saprotrophic	Grassland
Agaricus bisporus (Lange) Imbach	White Mushroom	Saprotrophic	Grassland
Cantharellus cibarius L.:Fries	Chanterelle	Mycorrhizal	*Pinus* sp.
Fistulina hepatica (Schaeff.:Fr)	Beefsteak Fungus	Parasitic	*Quercus pyrenaica* Willd.
Lactarius deliciosus (L.) Gray	Saffron Milk Cap	Mycorrhizal	*Pinus* sp.
Lactarius salmonicolor (Heim y Leclair)	Not known	Mycorrhizal	Mixed stands[a]
Lepista nuda (Bull. ex Fr.) Cooke	Wood Blewit	Saprotrophic	*Pinus* sp.
Leucopaxillus giganteus (Sowerby) Singer	Giant Leucopax	Saprotrophic	Grassland
Mycena rosea (Schumach.) Gramberg[b]	Rosy Bonnet	Saprotrophic	Mixed stands
Ramaria botrytis (Pers.:Fr.) Ricken	Cauliflower Coral	Mycorrhizal	*Quercus pyrenaica* Willd.
Russula delica (Fr.)	Milk-white Brittlegill	Mycorrhizal	Mixed stands
Sarcodon imbricatus (L.) P. Karst	Shingled Hedgehog	Mycorrhizal	*Pinus* sp.
Tricholoma portentosum (Fr.) Quél.	Dingy Agaric	Mycorrhizal	*Pinus* sp

[a]Refers to mixed stands of *Quercus* sp. and *Pinus sylvestris* Ait. [b]Species with unknown edibility; all the other are edible mushrooms.

Table 2. MIC values (μg/ml) of different antibiotics against Gram negative bacteria.

	E. coli		*K. pneumoniae*		*P. mirabilis*		*M. morgani*		*P. aeruginosa*		*A.baumannii*		*P. multocida*	
Ampicillin	>16	R	>16	R	≤8	S	>16	R		na		na		na
Amoxicillin/Clavulanic Acid	16/8	I	≤8/4	S	≤8/4	S	>16/8	R		na		na		na
Cephalothin	8	S	≤ 8	S	≤8	S	>16	R		na		na	≤8	S
Cefazolin	≤8	S	≤8	S	≤8	S	>16	R		na		na	≤8	S
Cefuroxime	≤4	S	≤4	S	≤4	S	>16	R		na		na	≤4	S
Cefoxitin	≤8	S	≤8	S	≤8	S	≤8	S		na		na	≤8	S
Cefotaxime	≤1	S	≤1	S	≤1	S	2	S	32	I	>32	R	≤1	S
Ceftazidime	≤1	S	≤1	S	≤1	S	2	S	2	S	16	I	≤1	S
Cefepime	16	S	≤1	S	≤1	S	≤1	S	8	S	>16	R	≤1	S
Nalidíxic Acid	>16	R		na	>16	R	≤16	S		na		na		na
Norfloxacin	>8	R	≤0.5	S	4	S	≤0.5	S	1	na		na		na
Ciprofloxacin	>2	R	≤0.5	S	>2	R	≤0.5	S	≤0.5	na	>2	R		na
Nitrofurantoin	≤32	S	≤32	S	>64	R	64	I		na		na		na
Fosfomycin	≤16	S	≤16	S	>64	R	>64	R	>64	na		na		na
Gentamicin	≤2	S	≤2	S	8	I	≤2	S	4	na	4	S		na
Imipenem	≤1	S	≤1	S	2	S	4	S	≤1	na		na		na
Piperacilin/Tazobactam	≤8	S	≤8	S	≤8	S	≤8	S	≤8	na		na		na
Trimethoprim/Sulfasoxazole	≤2/38	S	≤2/38	S	>4/76	R	>4/76	R		na	na	R		na
Tobramycin	≤2	S	≤2	S	4	S	≤2	S	≤2	na	≤2	S		na

S- Susceptible; I- Intermediate; R- Resistant (this classification was made according to the interpretative breakpoints suggested by Clinical and Laboratory Standards Institute-CLSI); na- not applicable.

Table 3. MIC values (μg/ml) of different antibiotics against Gram positive bacteria.

	S. epidermidis		*S. aureus*		MRSA		*S. saprophyticus*		*S. hominis-homin*		*S. agalactiae*		*S. pyogenes*		*E. faecalis*		*L. monocytogenes*	
Penicillin	8	Blac	8	Blac	>8	Blac	0.12	S	>8	Blac	≤0.03	S	0.25	S	2	S	0.25	S
Ampicillin	4	Blac	4	Blac	>8	Blac		na	>8	Blac		na		na	≤1	S	≤1	S
Cefoxitin Screen	>4	Pos	≤4	Neg	>4	Pos	≤4	Neg	>4	Pos		na		na		na		na
Oxacillin	>2	R	≤0.25	S	>2	R	≤0.25	S	>2	R		na		na		na		na
Clindamycin	≤0.25	S	≤0.25	S	1	I	≤0.25	S		na	≤0.25	S		na		na		na
Daptomycin	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S		na	≤0.5	S		na
Erythromycin	>4	R	≤0.25	S	≤0.25	S	>4	R	>4	R	≤0.25	S		na	2	I		na
Fosfomycin	≤32	S	≤32	S	≤32	S	≤32	S	≤32	S		na		na		na		na
Gentamicin	≤1	S	≤1	S	4	S	≤1	S	≤1	S		na		na		na		na
Ciprofloxacin	≤0.5	S	≤0.5	S	>2	R	≤0.5	S	2	I		na		na	1	S		na
Levofloxacin	≤1	S	≤1	S	>4	R	≤1	S	≤1	S	≤1	S		na	≤1	S		na
Linezolid	≤1	S	2	S	2	S	4	S	≤1	S	≤1	S		na	≤1	S		na
Synercid	≤0.5	S	≤0.5	S	1	S	≤0.5	S	≤0.5	S	≤0.5	S		na	>2	R		na
Teicoplanin	≤1	S	≤1	S	2	S	1	S	2	S		na		na	na	S		na
Tetracycline	≤1	S	≤1	S	≤1	S	>8	R	≤1	S	≤1	S		na	≤1	S		na
Trimethoprim/Sulfasoxazole	≤1/19	S	≤1/19	S	≤1/19	S	≤1/19	S	4/76	R		na		na		na		na
Vancomycin	≤1	S	≤1	S	2	S	≤1	S	≤1	S	≤1	S		na	2	S		na

S- Susceptible; I- Intermediate; R- Resistant (this classification was made according to the interpretative breakpoints suggested by Clinical and Laboratory Standards Institute-CLSI); Blac- Beta-lactamase positive; Pos- positive to Cefoxitin screening; Neg- negative to Cefoxitin screening; na- not applicable.

Table 4. MIC values (mg/ml) of the wild mushrooms extracts against clinical isolates of Gram negative bacteria.

	Escherichia coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Morganella morganni*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Pasteurella multocida*
Agaricus arvensis	> 20	> 20	> 20	> 20	> 20	> 20	20
Agaricus bisporus	> 20	> 20	> 20	> 20	> 20	> 20	20
Cantharellus cibarius	> 20	> 20	> 20	> 20	> 20	> 20	>20
Fistulina hepatica	20	> 20	> 20	20	> 20	> 20	20
Lactarius deliciosus	>20	> 20	> 20	> 20	> 20	> 20	> 20
Lactarius salmonicolor	> 20	> 20	> 20	> 20	> 20	> 20	>20
Lepista nuda	> 20	> 20	20	> 20	> 20	> 20	5
Leucopaxillus giganteus	20	> 20	20	> 20	> 20	> 20	10
Mycena rosea	> 20	> 20	20	> 20	> 20	> 20	>20
Ramaria botrytis	> 20	> 20	> 20	> 20	> 20	> 20	10
Russula delica	20	> 20	> 20	20	> 20	> 20	20
Sarcodon imbricatus	>20	> 20	> 20	> 20	> 20	> 20	>20
Tricholoma portentosum	> 20	> 20	> 20	> 20	> 20	> 20	10

Table 5. MIC values (mg/ml) of the wild mushrooms extracts against clinical isolates of Gram positive bacteria.

	Staphylococcus aureus	MRSA	*Staphylococcus epidermidis*	*Staphylococcus hominis*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Listeria monocytogenes*	*Streptococcus agalactiae*	*Streptococcus pyogenes*
Agaricus arvensis	> 20	>20	>20	>20	>20	> 20	> 20	> 20	10
Agaricus bisporus	>20	>20	>20	>20	>20	> 20	> 20	20	10
Cantharellus cibarius	> 20	>20	20	>20	> 20	> 20	20	10	20
Fistulina hepatica	10	10	20	20	>20	20	20	5	5
Lactarius deliciosus	> 20	20	> 20	>20	>20	> 20	>20	20	20
Lactarius salmonicolor	> 20	> 20	>20	>20	>20	>20	20	>20	10
Lepista nuda	> 20	>20	20	> 20	> 20	> 20	20	10	10
Leucopaxillus giganteus	> 20	>20	>20	>20	> 20	> 20	20	5	10
Mycena rosea	20	20	>20	>20	>20	> 20	20	>20	10
Ramaria botrytis	> 20	>20	> 20	>20	> 20	20	10	20	10
Russula delica	10	10	10	>20	20	20	20	5	5
Sarcodon imbricatus	20	20	> 20	>20	> 20	> 20	20	10	10
Tricholoma portentosum	20	20	20	>20	> 20	> 20	> 20	5	10

MRSA (Methicillin-resistant *Staphylococcus aureus*).

Table 6. MBC values (mg/ml) of the wild mushrooms extracts against three clinical isolates.

	Pasteurella multocida	*Streptococcus agalactiae*	*Streptococcus pyogenes*
Agaricus arvensis	20	-	10
Agaricus bisporus	20	20	10
Cantharellus cibarius	-	10	>20
Fistulina hepatica	20	10	5
Lactarius deliciosus	-	20	>20
Lactarius salmonicolor	-	-	10
Lepista nuda	5	20	10
Leucopaxillus giganteus	10	5	10
Mycena rosea	-	-	10
Ramaria botrytis	20	20	10
Russula delica	20	10	5
Sarcodon imbricatus	-	10	10
Tricholoma portentosum	10	10	10

(-) MIC > 20 mg/ml