Wild mushroom extracts potentiate the action of standard antibiotics against multi-resistant bacteria

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INTRODUCTION

The indiscriminate use of antibiotics and chemotherapeutic agents and the genetic ability of bacteria to transmit and acquire resistance resulted in the development of resistant species. In the last few years, several studies have been conducted in different countries to demonstrate the efficacy of natural products, not only studying their direct antimicrobial activity but also their capacity as resistance-modifying agents. The main objective of the present work was to evaluate the capacity of five wild mushroom extracts to potentiate the action of standard antibiotics, through synergisms that allow a decrease in their therapeutic doses and ultimately contribute to the reduction of resistances.

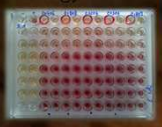
MATERIALS & METHODS

Mushroom species: Five mushroom species (*Fistulina hepatica, Leucopaxillus giganteus, Mycena rosea, Russula delica, Sarcodon imbricatum*) were collected in different ecosystems of the Trás-os-Montes region in the northeast of Portugal.

Microorganisms tested: Clinical isolates from patients hospitalized in various departments of the Hospital Center of Trás-os-Montes and Alto Douro – Chaves, Portugal.

Gram negative bacteria: *Escherichia coli* with different antibiotic resistance profile *E. coli 1* (resistant to Ampicillin, Ciprofloxacin and Trimethoprim/ Sulfamethoxazole), *E. coli 2* (resistant to Amoxicillin/Clavulanic acid and Ampicillin) and *E. coli ESBL* (resistant to Ampicillin, Nalidixic acid, Norfloxacin, Ciprofloxacin, Cephalosporins and Trimethoprim/Sulfamethoxazole) , isolated from urine.
Gram positive bacteria: *MRSA* (resistant to beta-lactams – Penicillin Ampicillin, Cefoxitin, but also to Quinolones – Ciprofloxacin and Levofloxacin) isolated from wound exudates;
All strains were identified using the MicroScan® panels automated methodology - Siemens.

Antimicrobial activity methology: Microdilution method and *p*-iodonitrotetrazolium chloride (INT) colorimetric assay.



MIC determination: 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 sample concentration that prevented the color change of the medium and exhibited an inhibition of microbial growth

Sinergistic Effect: Fractional inhibitory concentration (FIC) was calculated according to the equation: MIC(antibiotic+extract/MICantibiotic). The interpretations were made as follows: synergistic (S; <0.5), indifferent (I; 0.5 to 4), or antagonistic (A; >4). All the assays were carried out in duplicate.

RESULTS & DISCUSSION

Table 1: Effect of antibiotics individually and in combination with different mushroom extracts in MRSA (Methicillin-resistant *Staphylococcus aureus*).

Antibiotic	Antibiotic 100% MIC	Antibiotic 60% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 40% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 60% with *M. rosea* MIC/FIC (Effect)	Antibiotic 40% with *M. rosea* MIC/FIC (Effect)
Ampicillin	8	2.4/0.3 (S)	1.6/0.2 (S)	2.4/0.3 (S)	1.6/0.2 (S)
Cefoxitin	4	1.2/0.3 (S)	0.8/0.2 (S)	0.6/0.15 (S)	0.2/0.05 (S)
Ciprofloxacin	2	0.6/0.3 (S)	0.4/0.2 (S)	0.6/0.3 (S)	0.4/0.2 (S)
Levofloxacin	4	1.2/0.3 (S)	0.8/0.2 (S)	2.4/0.6 (I)	0.8/0.2 (S)
Penincillin	8	2.4/0.3 (S)	1.6/0.2 (S)	2.4/0.3 (S)	0.8/0.1 (S)
Antibiotic	**Antibiotic 100% MIC**	**Antibiotic 60% with *R. delica* MIC/FIC (Effect)**	**Antibiotic 40% with *R. delica* MIC/FIC (Effect)**	**Antibiotic 60% with *S. imbricatum* MIC/FIC (Effect)**	**Antibiotic 40% with *S. imbricatum* MIC/FIC (Effect)**
Ampicillin	8	4.8/0.6 (I)	3.2/0.4 (S)	>4.8	>3.2
Cefoxitin	4	1.2/0.3 (S)	0.4/0.1 (S)	nt	nt
Ciprofloxacin	2	>1.2	>0.8	>1.2	>0.8
Levofloxacin	4	1.2/0.3 (S)	0.8/0.2 (S)	0.6/0.15 (S)	0.4/0.1 (S)
Penincillin	8	4.8/0.6 (I)	1.6/0.2 (S)	>4.8	>3.2

Table 2: Effect of antibiotics individually and in combination with different mushroom extracts in *Escherichia coli 1*

Antibiotic	Antibiotic 100% MIC	Antibiotic 60% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 40% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 60% with *L. giganteus* MIC/FIC (Effect)	Antibiotic 40% with *L. giganteus* MIC/FIC (Effect)
Ampicillin	16	9.6/0.6 (I)	6.4/0.4 (S)	4.8/0.3 (S)	0.8/0.05 (S)
Amoxicillin/Clavulanic acid	16	>9.6	>6.4	>9.6	6.4/0.4 (S)
Ciprofloxacin	2	nt	nt	nt	nt
Trimethoprim/Sulfamethoxazole	76	nt	nt	nt	nt
Antibiotic	**Antibiotic 100% MIC**	**Antibiotic 60% with *R. delica* MIC/FIC (Effect)**	**Antibiotic 40% with *R. delica* MIC/FIC (Effect)**		
Ampicillin	16	9.6/0.6 (I)	3.2/0.2 (S)		
Amoxicillin/Clavulanic acid	16	9.6/0.6 (I)	3.2/0.2 (S)		
Ciprofloxacin	2	nt	nt		
Trimethoprim/Sulfamethoxazole	76	Nt	nt		

Table 3. Effect of antibiotics individually and in combination with different mushroom extracts in *Escherichia coli 2*.

Antibiotic	Antibiotic 100% MIC	Antibiotic 60% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 40% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 60% with *L. giganteus* MIC/FIC (Effect)	Antibiotic 40% with *L. giganteus* MIC/FIC (Effect)
Ampicillin	16	nt	nt	>9.6	>6.4
Amoxicillin/Clavulanic acid	16	nt	nt	nt	nt
Ciprofloxacin	2	nt	nt	>1.2	>0.8
Trimethoprim/Sulfamethoxazole	76	nt	nt	>45.6	>30.4
Antibiotic	**Antibiotic 100% MIC**	**Antibiotic 60% with *R. delica* MIC/FIC (Effect)**	**Antibiotic 40% with *R. delica* MIC/FIC (Effect)**		
Ampicillin	16	4.8/0.3 (S)	1.6/ 0.1 (S)		
Amoxicillin/Clavulanic acid	16	nt	nt		
Ciprofloxacin	2	0.6/0.3 (S)	0.2/0.1 (S)		
Trimethoprim/Sulfamethoxazole	76	45.6/0.6 (I)	15.2/0.2 (S)		

Table 4. Effect of antibiotics individually and in combination with different mushroom extracts in Extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli*.

Antibiotic	Antibiotic 100% MIC	Antibiotic 60% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 40% with *F. hepatica* MIC/FIC (Effect)	Antibiotic 60% with *L. giganteus* MIC/FIC (Effect)	Antibiotic 40% with *L. giganteus* MIC/FIC (Effect)
Ampicillin	16	>9.6	>6.4	4.8/0.3 (S)	3.2/0.2 (S)
Amoxicillin/Clavulanic acid	16	nt	nt	nt	nt
Ciprofloxacin	2	>1.2	>0.8	1.2/0.6 (I)	0.4/0.2 (S)
Trimethoprim/Sulfamethoxazole	76	>45.6	>30.4	22.8/0.3 (S)	15.2/0.2 (S)
Antibiotic	**Antibiotic 100% MIC**	**Antibiotic 60% with *R. delica* MIC/FIC (Effect)**	**Antibiotic 40% with *R. delica* MIC/FIC (Effect)**		
Ampicillin	16	4.8/0.3 (S)	1.6/0.1 (S)		
Amoxicillin/Clavulanic acid	16	nt	nt		
Ciprofloxacin	2	0.6/0.3 (S)	0.2/0.1 (S)		
Trimethoprim/Sulfamethoxazole	76	22.8/0.3 (S)	7.6/0.1 (S)		

✓ The results obtained showed higher synergistic effects against MRSA (**Table 1**) than against *E. coli* (**Tables 2-4**).
✓ Regarding MRSA (**Table 1**), *Mycena rosea* and *Fistulina hepatica* were the best extracts for synergistic effects
✓ *Mycena rosea* and *Fistulina hepatica* extracts allowed synergistic effects with quinolones (ciprofloxacin and levofloxacin)(**Table 1**).
✓ It can be observed in all the extracts, an increase of FIC values with the increase of antibiotic percentage, occurring in some cases an increase of FIC higher than 0.5, and disappearing the synergistic effect. Nevertheless, for *Fistulina hepatica* extract, despite the increase of FIC values with higher antibiotic percentage, the synergism still remains (**Table 1**).
✓ *Sarcodon imbricatum* extract gave the worst results and did not show synergisms with the tested antibiotics except for levofloxacin (**Table 1**).
✓ The efficiency of *Russula delica* extract against *E. coli* (**Tables 2** and **3**) was notoriously higher than *Leucopaxillus giganteus* extract; nevertheless the latter extract showed better synergistic effects against ESBL *E. coli* (**Table 4**).
✓ Among the three mushroom species, *Fistulina hepatica* extract gave the lowest synergistic effect against *E. coli* (**Tables 2 and 3**).
✓ The action of ciprofloxacin was potentiated by *Russula delica* or *Leucopaxillus giganteus* extracts (**Tables 3 and 4**).
✓ *Russula delica* extract was the only one that gave synergistic effects with the antimetabolic antibiotic trimethoprim/sulfamethoxazole (**Tables 3 and 4)**

CONCLUSIONS

The present study shows that, similarly to plants, some mushroom extracts can potentiate the action of antibiotics extensively used in clinical practice for Gram-positive or Gram-negative bacteria, and might be used against multi-resistant bacteria.

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