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

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Running Head: Mushroom extracts potentiate the action of antibiotics

Abstract

Aims: The main objective of the present work was to evaluate the capacity of 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.

Methods and Results: Wild mushroom extracts were applied to different multi-resistant microorganisms (*Escherichia coli*, Extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA), combined with commercial antibiotics (Penicillin, Ampicillin, Amoxicillin/Clavulanic acid, Cefoxitin, Ciprofloxacin, Cotrimoxazol, Levofloxacin). Microdilution method was used to determine minimum inhibitory concentrations (MICs). The results obtained showed higher synergistic effects against MRSA than against *E. coli*. *Mycena rosea* and *Fistulina hepatica* were the best extracts for synergistic effects against MRSA. The efficiency of *Russula delica* extract against *E. coli 1* (resistant to Ampicillin, Ciprofloxacin and Trimethoprim/Sulfasoxazole) and *E. coli 2* (resistant to Amoxicillin/Clavulanic acid and Ampicillin) was higher than that of *Leucopaxillus giganteus* extract; nevertheless the latter extract exhibited better synergistic effects against ESBL *E. coli*.

Conclusions: This 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, with positive action even against multi-resistant bacteria.

Significance and Impact of the Study: Mushroom extracts could decrease therapeutic doses of standard antibiotics and reduce microorganism's resistance to those drugs.

Keywords: Antibiotics; Wild mushroom extracts; Synergism; Multi-resistant bacteria

Introduction

The indiscriminate use of antibiotics and chemotherapeutic agents, among other factors, has been contributing for the development of resistant species (Andrade *et al.* 2006). For patients, antimicrobial resistance increases morbidity and mortality, while there is a significant increase in costs for health care institutions. Because of that, a huge effort has been directed towards controlling antibiotic use and raising public awareness of the need for prudent use of antibiotics (Dancer 2001; Coutinho *et al.* 2005) and finding new sources of active molecules.

In general, bacteria serve as host for multiple genetic elements like genes, integrons, transposons and plasmids that confer antibiotic resistance phenotypes. So, they have the genetic ability to transmit and acquire resistance to drugs that are used as therapeutic agents (Santos *et al.* 2011).

Bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* are microorganisms of concern with regard to multi-resistances (Calbo *et al.* 2006; Donskey 2006; Chambers and Deleo 2009). Due to this problem, there is a need to investigate new compounds or strategies to reverse this tendency in order to achieve the appropriate and effective treatment against infections by such microorganisms.

Natural matrices, in particular wild mushroom extracts emerge as interesting possibilities to be explored as antimicrobial drugs (Alves *et al.* 2012a). Mushrooms are rich sources of natural antimicrobials, as they produce antibacterial and antifungal compounds to survive in their natural environment (Rathee *et al.* 2012).

Additionally, it has been reported that the exposure of some food pathogens to increasing sublethal amounts of natural antimicrobials resulted in no significant global

effects on the acquisition of direct tolerance, namely by *L. monocytogenes*, which maybe an additional advantage when compared to standard antibiotics (Luz *et al.* 2012). In the last few years, a number of 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 (Benoit-Vical *et al.* 2006; Singh *et al.* 2007). The resistance modification is based on creating a synergistic relationship, i.e. a positive interaction created when two combined agents exert an inhibitory effect that is greater than the sum of their individual effects (Aiyegoro and Okoh 2009). It has been proven that, in addition to production of intrinsic antimicrobial compounds, plants also produce multi-drug resistance inhibitors, which enhance the activity of the antimicrobial compounds (Stermitz *et al.* 2000).

According to Wagner and Ulrich-Merzenich (2009), natural extracts can provide a synergistic relationship with antibiotics, through a multi-or single-target action. A "Synergistic multi-target effect" means that the single constituents of a mono-extract or a multi-extract combination affect not only one single target, but several targets, and therefore cooperate in an agonistic, synergistic way. On the other hand, the extracts can display a mono-target action that results from the interaction of the extract with one single target. Several other authors support the mentioned idea reporting studies of synergism between natural products and antibiotics (Coutinho *et al.* 2008; Hemaiswarya *et al.* 2008; Aiyegoro and Okoh 2009; Coutinho *et al.* 2009; Palaniappan and Holley 2010; Braga *et al.* 2011; Santos *et al.* 2011; Mitchell *et al.* 2012).

The understanding of the molecular mechanisms of synergy would tackle a new strategy for the treatment of infectious diseases, overcome drug-resistant pathogens, and reduce the use of antibiotics and consequently the side effects created by them (Hemaiswarya *et al.* 2008).

Taking into account our previous results highlighting the antibacterial activity of extracts from specific wild mushrooms (*Fistulina hepatica*, *Leucopaxillus giganteus*, *Mycena rosea*, *Russula delica*, *Sarcodon imbricatum*) (Alves *et al.* 2012b), those extracts were applied to different multi-resistant microorganisms (*Escherichia coli*, Extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA), combined with commercial antibiotics (Penicillin, Ampicillin, Amoxicillin/Clavulanic acid, Cefoxitin, Ciprofloxacin, Cotrimoxazol, Levofloxacin). The main objective was to evaluate the capacity of natural extracts to potentiate the action of standard antibiotics, through synergisms that allow a decrease in their therapeutic doses and a protection against increasing resistance. Furthermore, this is an important study since the available reports on literature are mainly related with plants and not with mushroom extracts.

Materials and methods

Mushroom species and extracts preparation

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 according to the information provided in Alves *et al.* (2012b). 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, the Netherlands) and kept in the dark in hermetically sealed plastic bags up to the point of analysis.

Each mushroom lyophilized sample (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 °C under reduced pressure to remove methanol (rotary evaporator Büchi R-210, Flawil, Switzerland), lyophilized, redissolved in water, at a defined concentration and stored at 20 °C for further use.

Antibiotics

The antibiotics were selected according to the resistance profile of the studied bacteria: Penicillin, Ampicillin, Amoxicillin/Clavulanic acid, Cefoxitin, Ciprofloxacin, Cotrimoxazol, Levofloxacin and were used as antibiotic (Sigma-Aldrich, St Quentin Fallavier, France).

Microorganisms

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. One Gram-positive bacteria, MRSA (resistant to beta-lactams − Penicillin Ampicillin, Cefoxitin, but also to Quinolones − Ciprofloxacin and Levofloxacin) isolated from wound exudates; and three Gram-negative bacteria 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, were used to screen the antimicrobial activity of the mushroom extracts and antibiotics. All strains were identified using the MicroScan® panels automated methodology – Siemens (Camberley, UK).

Bacterial susceptibility determinations

MIC determinations were performed by the microdilution method and the rapid INT (*p*-iodonitrotetrazolium chloride) colorimetric assay following the methodology previously described by the authors (Alves *et al.* 2012b). Initially, four dilutions were made with each mushroom extract and antibiotic in MHB (Mueller Hinton broth obtained from Biomerieux, Marcy l'Etoile, France), with a final concentration equivalent to their MICs: 20 mg/mL for extracts, previously reported in Alves *et al.* (2012b) and the concentrations stated in **Tables 1** and **2** for antibiotics.

Each antibiotic was tested individually or in combination with the mushroom extract in two different percentages: 40% of antibiotic (20 µL of antibiotic + 30 µL of extract) and 60% of antibiotic (30 µL of antibiotic + 20 µL of extract).

Dilutions were carried out over the wells containing 450 µL of MHB and afterwards, 10 µL of inoculum (1×10^8 CFU/mL) was added to all the wells. One negative (only with MHB) and one positive (with MHB and the inoculum) control were performed. The plates were incubated at 37 °C, for 24 h, in an oven (Jouan, Berlin, Germany). The MICs of the samples were visualized following the addition of INT dye (Sigma-Aldrich, St Louis, MO, USA) (0.2 mg/mL, 40 µL) and incubation at 37 °C for 30 min. Viable microorganisms reduced the yellow dye to a pink color. MIC was defined as the lowest sample concentration that prevented the color change of the medium and exhibited an inhibition of microbial growth. Fractional inhibitory concentration (FIC) was calculated according to the equation:

$MIC_{antibiotic+extract}/MIC_{antibiotic}$). The interpretations were made as follows: synergistic (S; <0.5), indifferent (I; 0.5 to 4), or antagonistic (A; >4) (Fankam *et al.* 2011). All the assays were carried out in duplicate.

Results

Taking into account our previous findings related to antibacterial activity of wild mushrooms (Alves *et al.* 2012b), the extracts that showed higher activity were selected to evaluate possible synergistic effects with different antibiotics. *Fistulina hepatica* (MIC=10 mg/mL), *Russula delica* (MIC=10 mg/mL), *Mycena rosea* (MIC=20 mg/mL) and *Sarcodon imbricatum* (MIC=20 mg/mL) extract gave the highest antimicrobial activity against MRSA, while *Fistulina hepatica*, *Leucopaxillus giganteus* and *Russula delica* extracts (MICs=20 mg/mL) were the best ones against *E. coli* (Alves *et al.* 2012b). It should be stated that *Mycena rosea* and *Sarcodon imbricatum* extracts (up to 20 mg/mL) did not show activity against *E. coli*, while *Leucopaxill*us *giganteus* (up to the same concentration) did not show antibacterial activity for MRSA.

The results obtained showed higher synergistic effects between mushroom extracts and standard antibiotics for MRSA (**Table 1**) than for *E. coli* (**Tables 2-4**).

Regarding MRSA (**Table 1**), *Mycena rosea* and *Fistulina hepatica* were the best extracts for synergistic effects. Both extracts gave synergisms with β-lactamic antibiotics (penicillin, ampicillin and cefoxitin). The combination of *Mycena rosea* with cefoxitin 40% revealed the lowest FIC value (0.05; highest synergism), followed by its combination with penicillin at the same percentage (FIC=0.1).

Mycena rosea and *Fistulina hepatica* extracts also 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**). *Sarcadon imbricatum* extract gave the worst results and did not show synergisms with the tested antibiotics, except for levofloxacin (**Table 1**).

Three *E. coli* strains with different antibiotics resistance profiles were also studied; one of them was extended-spectrum beta-lactamase-producing (ESBL).

The efficiency of *Russula delica* extract against *E. coli* (**Tables 2** and **3**) was higher than *Leucopaxillus giganteus* extract; nevertheless the latter extract showed better synergistic effects against ESBL *E. coli* (**Table 4**). Therefore, studies should be carried out in order to clarify if *Leucopaxillus giganteus* inhibits β-lactamases production or may act as a beta-lactamase inhibitory compound. Among the three mushroom species, *Fistulina hepatica* extract gave the lowest synergistic effect against *E. coli* (**Tables 2 and 3**).

The action of ciprofloxacin (quinolone) 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)**. As far as we know, this is the first study reporting synergism between natural matrices and the mentioned antibiotic.

Discussion

The fight against multi-resistant bacteria is a worldwide problem of public health that should be considered in several aspects. The knowledge about the processes related to antibiotics action and resistance development, the synthesis and pharmacological evaluation of new and more potent antimicrobial agents, their subsequent therapeutic

application in a rational way, and the adoption of procedures to control hospital infections, represent different levels of continuous and interlinked actions (Silveira *et al.* 2005). Furthermore, the new research strategies on antimicrobial natural products involving unexplored matrices and the use of genomic tools to increase their production, might accelerate the discovery of new antibiotics, overcoming the rapid development of bacteria resistance to the available therapeutic agents (Guimarães *et al.* 2010).

Several successful studies have been carried out in order to find new natural or synthetic products with antimicrobial activity and lower toxicity for hosts (Anaisse 1992; Edwards and Filler 1992; Graybill 1992; Guimarães *et al.* 2010).

Fistulina hepatica, *Leucopaxillus giganteus*, *Mycena rosea*, *Russula delica* and *Sarcodon imbricatum* extracts are good examples (Alves *et al.* 2012b) and, in the present study, they were applied to different multi-resistant microorganisms (*Escherichia coli*, Extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA), combined with commercial antibiotics (Penicillin, Ampicillin, Amoxicillin/Clavulanic acid, Cefoxitin, Ciprofloxacin, Cotrimoxazol, Levofloxacin).

The results obtained showed higher synergistic effects against MRSA than against *E. coli*. *Mycena rosea* and *Fistulina hepatica* were the best extracts for synergistic effects against MRSA (methicillin-resistant *Staphylococcus aureus*). The synergistic effects against MRSA were observed for some β-lactamics (penicillin, ampicillin and cefoxitin) and for two quinolones (ciprofloxacin and levofloxacin). Liu *et al.* (2000) also reported synergism against MRSA of a combination between baicalin, a natural compound from *Soutellaria amoena*, and β-lactamic antibiotics such as ampicillin. The mentioned compound increased β-lactamics efficiency through inhibition of β-lactamases production. In other study, Zhao *et al.* (2001) concluded that combination of ECG

(epigallocatechin gallate), from *Camellia sinensis*, with ampicillin also lead to synergisms against MRSA, related to a similar interaction with peptidoglycan, in which β-lactamics normally act. Braga *et al.* (2011) described synergism against MRSA due to a combination of pomegranate extract and ciprofloxacin, which blocked the efflux bombs. Other authors (Bazzaz *et al.* 2008; An *et al.* 2011) also reported synergistic effects of plant extracts and quinolones (ciprofloxacin and levofloxacin) against MRSA.

The efficiency of *Russula delica* extract against *E. coli 1* (resistant to Ampicillin, Ciprofloxacin and Trimethoprim/Sulfasoxazole) and *E. coli 2* (resistant to Amoxicillin/Clavulanic acid and Ampicillin) was higher than that of *Leucopaxillus giganteus* extract; nevertheless the latter extract exhibited better synergistic effects against ESBL (extended-spectrum beta-lactamase-producing) *E. coli.* Darwish and Aburjai (2010) also described that two plants, *Anagyris foetidae* and *Lepidium sativum*, had antagonistic effect upon amoxicillin activity against a standard *E. coli* strain (without resistances), but conducting to synergistic effects against ESBL *E. coli.*

As is in the present study in which ciprofloxacin was potentiated by *Leucopaxillus giganteus* and *Russula delica* extracts, Natarajan et al. (2008) showed synergisms between *Humulus lupulus* extract and ciprofloxacin against *E. coli*, suggesting the modification of membrane permeability as possible mechanism of action.

The number of available studies regarding synergisms between natural products and β-lactamics seems to be much lower than other antibiotics (Coutinho *et al.* 2008; Coutinho *et al.* 2009; D'Arrigo *et al.* 2010; Braga *et al.* 2011; Sousa *et al.* 2013).

Overall, 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.

This is a pioneer study regarding synergistic effects between mushroom extracts and antibiotics that could allow a decrease in the therapeutic doses and a protection against increasing microorganism's resistance. Nevertheless, more studies should be conducted in order to clarify the mechanisms of action that support the observed effects upon each antibiotic and microorganism.

Conflict of Interest

The authors have no conflicts of interest.

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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

MIC- Minimal inhibitory concentration (μg/mL); FIC- Fractional inhibitory concentration; (S)- Synergism; (I)- Indifference; nt- not tested

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		

MIC- Minimal inhibitory concentration (μg/mL); FIC- Fractional inhibitory concentration; (S)- Synergism; (I)- Indifference; nt- not tested

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)		

MIC- Minimal inhibitory concentration (μg/mL); FIC- Fractional inhibitory concentration; (S)- Synergism; (I)- Indifference; nt- not tested.

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)		

MIC- Minimal inhibitory concentration (μg/mL); FIC- Fractional inhibitory concentration; (S)- Synergism; (I)- Indifference; nt- not tested.