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Synergistic Activity of the Antibiotic Meropenem in Combination with Edible Mushroom Extracts against Multidrug Resistant Bacteria

Debasmita Chatterjee[1], Dipankar Halder[1] and Satadal Das[2*]

[1]Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-700032, India.
[2]Brucella Research Laboratory, Peerless Hospital and B. K. Roy Research Centre, Kolkata- 700094, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SD and DH designed the study. Author DC managed the literature searches, wrote the protocol and wrote the first draft of the manuscript, analyses of the study, performed the spectroscopy analysis. All authors managed the experimental process and author DC identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of this study was to evaluate the antibacterial potency of meropenem in synergism with five edible mushroom extracts against multidrug resistant clinical isolates.
Methodology: Minimum Inhibitory Concentration (MIC) values of meropenem in combination with each of the extract of mushrooms *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus eous*, *Pleurotus sajorcaju*, *Calocybe indica* was determined separately against multidrug resistant bacterial strains. Among the applied microbial strains four were extended spectrum β lactamase (ESBL) producing *Escherichia coli*, one each of ESBL producing *Klebsiella pneumoniae* and *Acinetobacter baumannii*. The synergistic antibacterial activity was assessed and compared based on Fractional Inhibitory Concentration (FIC) index.

**Corresponding author: E-mail: drsatdas@hotmail.com;*

Results: The extracts of the *P. eous* and *P. ostreatus* showed excellent synergistic activity, against all the strains of ESBL producing *E. coli* as well as ESBL producing *Klebsiella pneumoniae,* whereas the extract of mushroom *P. florida* and *C. indica* showed synergistic effect against some of the ESBL producing microorganisms. This study indicated edible mushrooms extracts can potentiate the action of standard antibiotic meropenem against multidrug resistant bacteria.
Conclusion: Synergistic effect of conventional antibiotic meropenem with edible mushroom extracts against multidrug resistant microorganisms will help in fighting against multidrug resistant bugs in future.

Keywords: Synergism; edible mushrooms; meropenem; ESBL microorganisms.

1. INTRODUCTION

Multidrug resistance activity of bacteria is responsible for many critical infections posing a therapeutic challenge in the treatment of hospitalized as well as community based infected patients [1]. Extended spectrum β lacatamase (ESBL) producing microorganisms show resistance against most of the third generation cephalosporins and aztreonam and hence carbapenem is the drug of choice against those microorganisms. But the most menacing fact is that carbapenem resistant isolates have already been reported [1]. The total number of characterized ESBL groups has now exceeded 200 and a website with the nomenclature and classification of ESBLs has already been hosted by George Jacoby and Karen Bush [1]. Bacteria possessing the antibiotic resistant genes propagate at a rapid rate, and therefore, very limited therapeutic agents are available to treat them [2]. Therefore, there is an urgent need to evolve newer strategies against these resistant bugs to achieve an effective treatment against the drug resistant microorganisms causing infections.

Mushroom is being considered as a potent "green" source of different antimicrobial agents to combat such grave infections [3,4]. Mushrooms possess many bioactive components which causes various health beneficial effects such as immune system modulation, biological response modification, etc. [5]. It has been noticed that there is a recent rise in interest among scientists regarding mushrooms not only as a healthy food but also for its bioactive compounds which are getting used as complementary medicine or dietary supplements for their anticancer, antiviral, immune stimulating, hypocholesterolemic and hepatoprotective activities [6]. Various wild and medicinal mushrooms extracts showed broad spectrum antibacterial activities against common pathogenic gram negative and positive bacteria [7]. Bioactive compounds such as terpenes, benzoic acid derivative, organic acids, proteins, ribonuclease may be responsible for this kind of antimicrobial activity [7,8]. According to Wagner, natural extracts can share a synergistic relationship with antibiotics and several other authors had also supported this idea [9,10]. Synergism of the bioactive components of extract such as flavonoids and essential oils in combination with synthetic drugs can combat not only bacterial, fungal but also mycobacterial infections. The combined mode of action differs significantly than same drugs acting alone [11]. Previous study on synergism of clove oil components such as eugenol and β-carophyllene was evaluated against oral bacteria in combination with antibiotics such as ampicillin or gentamicin. The study reported the lowering of MIC and MBC to one half to one sixteenth as a result of combination of clove oil or eugenol with antibiotics [12]. Similarly, another study reported the synergistic interaction between the essential oils of endemic Moroccan thyme species such as *Thymus maroccanus* and *T. broussonetii* with antibiotics ciprofloxacin, gentamicin, pristinamycin and cefexime against antibiotic resistant bacteria responsible for nosocomial infections. The result showed that out of eighty combinations tested between essential oils and antibiotics, 71% showed full synergism, 20% showed partial synergism and 9% showed no effect. The study showed that the effect on gram positive bacteria was much more in comparison to gram negative bacteria [13]. Therefore synergism has beneficial effects in terms of lowering the gradual declination in efficacy of single drug, over long term use, and this can be a new approach to reduce the rate of antibiotic resistance showed by the microorganisms. There are various advantages of the combinatorial approach such as it increases the potency, reduces side effects, reduces the time of cure, provides broad spectrum activity than single therapeutic use [10].

In the present study, we have evaluated the antimicrobial potency of the extracts of five edible mushrooms, namely *P. ostreatus*, *P. florida*, *P. eous*, *P. sajorcaju*, and *C. indica* in combination with standard antibiotic meropenem against four ESBL positive *Escherichia coli*, one ESBL positive *Klebsiella pneumoniae* and one multidrug resistant *Acinetobacter baumannii.* The main objective of this study was to observe whether these extracts can lower the therapeutic dose of the antibiotic or not. In turn it may lower the chances of development of drug resistance after prolong use. As, no study is available regarding the synergistic effect of standard antimicrobial agent with extracts of edible mushrooms against multidrug resistant bacteria, the present study can be considered as a significant contribution to the existing literature available.

2. MATERIALS AND METHODS

2.1 Collection of Mushroom Species and the Process of Extraction

Edible species of *P. ostreatus, P. florida, P. eous, P. sajorcaju,* and *C. indica* were collected from mushroom cultivation farm of Ramakrishna Mission, Narendrapur, West Bengal, India. Samples were collected and shade dried under the sun for about 72 hours and then powdered for long term storage in dry conditions.

In a sample batch 10 g of powdered sample was extracted with 100 mL of 60% (v/v) aqueous ethanol, for 72 hours. Then the extract was passed through Whatman filter paper No. 1. The clear filtrate was again filtered with 0.22 micron syringe filter and the content was lyophilized and stored at 4℃.

2.2 Microorganisms

Four ESBL producing strains of *E. coli*, one ESBL producing strain of *K. pneumoniae* and one strain of *A. baumannii* were isolated from various clinical samples from patients admitted in Intensive Coronary Care Unit (ICCU) Peerless Hospital, Kolkata, India. The microorganisms were identified by standard laboratory techniques and by automated panels of system Vitek ® 2 (Biomeiurieux, USA).

2.3 Antibiotic

Meropenem (AstraZeneca, Macclesfield, Cheshire, UK) was used for the assay.

2.4 Antibiotic Resistance Profile of the Test Strains

The strains were tested for their antibiotic sensitivity profile by disc diffusion method. Antibiotic discs were purchased from Hi-Media Laboratory Ltd., Mumbai (India) [14]. Drug resistance pattern of the selected strains has been tabulated in the Table 1.

2.5 Antimicrobial Assays

MIC of the mixture of meropenem solution and mushroom extracts (1:1, v/v) was assessed. The final stock concentrations of the extracts were maintained at 25 mg/mL and that of meropenem at 0.05 mg/mL. Serial dilutions were carried out with Mueller Hinton broth in a 96 well microtitre plate. 10 µL of 0.5 MacFarland opacity cultures were used for the assay. One initial absorbance was measured immediately after the addition of culture and the final one was measured after an overnight incubation at 37℃ by 96 well plate

Table 1. Antibiotic resistance profile of the collected strains of microorganisms

Strain designation	Resistance profile
Escherichia coli strain 1	Augmentin, Aztreonam, Ceftazidime, Ciprofloxacin, Ceftriaxone, Cefuroxime, Cefotaxime, Co-Trimoxazole, Cefepime, Nalidixic acid,
Escherichia coli strain 2	Augmentin, Aztreonam, Cefoperazone, Ceftazidime, Ceftriaxone, Cefuroxime, Cefotaxime, Ciprofloxacin, Co-Trimoxazole, Levofloxacin, Nalidixic acid, Nitrofurantoin, Netilmicin, Sparfloxacin, Cefepime.
Escherichia coli strain 3	Augmentin, Azithromycin, Furazolidone, Ceftriaxone, Cefuroxime, Cefotaxime, Ciprofloxacin, Gentamicin, Nalidixic acid, Ofloxacin, Sparfloxacin, Cefepime.
Escherichia coli strain 4	Augmentin, Azithromycin, Cefuroxime, Cefotaxime, Ciprofloxacin, Co-Trimoxazole, Nalidixic acid, Ofloxacin, , Sparfloxacin, Cefepime
Klebsiella pneumoniae strain 1	Augmentin, Aztreonam, Ceftazidime, Ciprofloxacin, Co-Trimoxazole, Nalidixic acid, Ofloxacin, Cefepime, Sparfloxacin
Acinetobacter baumannii	Ampicillin, Amikacin, Amoxyclav, Cefotaxime, Ceftazidime, Co-Trimoxazole, Ticarcillin-clav, Cefepime, Ciprofloxacin.

reader (Erba LisaScan II Tranasia Mannheim, Germany). The Fractional inhibitory concentration (FIC) was calculated according to the equation:

$$MIC_{antibiotic + extract} / MIC_{antibiotic}$$ [2].

Interpretation was carried out as follows: (<1) Synergistic; (1-4) Indifference; (>4) Antagonistic. All the assays were carried out in duplicate [10].

3. RESULTS

The extracts of mushroom *P. ostreatus* and *P. eous* showed excellent synergistic activity based on the FIC index, against all the strains of ESBL positive *E. coli* and *K. pneumoniae* and also reduced the MIC value of the meropenem by various fold (Table 2 and Table 3). However, none of the extracts showed synergistic relationship with meropenem against multidrug resistant *Acinetobacter baumannii*. The extracts of mushroom *P. florida* showed synergistic effect against four of the ESBL positive microorganisms namely three strains of *E. coli* and one strain of *K. pneumoniae* (Table 4). The extract of *C. indica* showed synergistic activity with meropenem against three strains of *E. coli* and antagonistic activity against *K. pneumoniae* and *A. baumannii* (Table 5). The extract of mushroom *P. sajor caju* did not show any kind of synergistic activity, against any microorganisms and they appeared antagonistic against the ESBL positive microorganisms (Table 6). The extracts of all the other mushrooms screened potentiated the activity of the antibiotic meropenem up to eight fold. Thus this is an encouraging result to mitigate the resistance against meropenem to treat moribund patients critically infected with ESBL positive microorganisms.

Table 2. Effects of antibiotic alone and in combination with *P. ostreatus* extracts against all the multidrug resistant strains

Microorganisms	Antibiotic 100% MIC (µg/mL)	Antibiotic 50% with *P. ostreatus* extract MIC (µg/mL)	Action based on FIC index
Escherichia coli ESBL positive; strain 1	0.4	6.25	Antagonistic
Escherichia coli ESBL positive; strain 2	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 3	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 4	12.5	3.125	Synergy
Klebsiella pneumoniae ESBL positive; strain 1	25	6.25	Synergy
Acinetobacter baumannii	0.4	6.25	Antagonistic

Table 3. Effects of antibiotic alone and in combination with *P. eous* extracts against all the multidrug resistant strains

Microorganisms	Antibiotic 100% MIC (µg/mL)	Antibiotic 50% with *P. eous* extract MIC (µg/mL)	Action based on FIC index
Escherichia coli ESBL positive; strain 1	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 2	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 3	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 4	12.5	3.125	Synergy
Klebsiella pneumoniae ESBL positive; strain 1	25	3.125	Synergy
Acinetobacter baumannii	0.4	3.125	Antagonistic

Table 4. Effects of antibiotic alone and in combination with *P. florida* extracts against all the multidrug resistant strains

Microorganisms	Antibiotic 100% MIC (µg/mL)	Antibiotic 50% with *P. florida* extract MIC (µg/mL)	Action based on FIC index
Escherichia coli ESBL positive; strain 1	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 2	0.4	6.25	Antagonistic
Escherichia coli ESBL positive; strain 3	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 4	12.5	3.125	Synergy
Klebsiella pneumoniae ESBL positive; strain 1	25	3.125	Synergy
Acinetobacter baumannii	0.4	6.25	Antagonistic

Table 5. Effects of antibiotic alone and in combination with *C. indica* extracts against all the multidrug resistant strains

Microorganisms	Antibiotic 100% MIC (µg/mL)	Antibiotic 50% with *C. indica* extract MIC (µg/mL)	Action based on FIC index
Escherichia coli ESBL positive; strain 1	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 2	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 3	0.4	0.195	Synergy
Escherichia coli ESBL positive; strain 4	12.5	25	Indifference
Klebsiella pneumoniae ESBL positive; strain 1	25	25	Indifference
Acinetobacter baumannii	0.4	25	Antagonistic

Table 6. Effects of antibiotic alone and in combination with *P. sajor-caju* extracts against all the multidrug resistant strains

Microorganisms	Antibiotic 100% MIC (µg/mL)	Antibiotic 50% with *P. sajor-caju* extract MIC (µg/mL)	Action based on FIC index
Escherichia coli ESBL positive; strain 1	0.4	6.25	Antagonistic
Escherichia coli ESBL positive; strain 2	0.4	6.25	Antagonistic
Escherichia coli ESBL positive; strain 3	0.4	6.25	Antagonistic
Escherichia coli ESBL positive; strain 4	12.5	25	Indifferent
Klebsiella pneumoniae ESBL positive; strain 1	25	25	Indifferent
Acinetobacter baumannii	0.4	3.125	Antagonistic

4. DISCUSSION

Previously various other studies have reported the antimicrobial activity of different medicinal plants against multidrug resistant strains [15]. A study reported the synergistic interaction of extracts of *Acorus calamus*, *Holarrhena antidysenterica* and *D. regia* with two antibacterial drugs tetracycline and ciprofloxacin against ESBL producing *Escherichia coli* [14]. Previously Alves et al. [2] reported the action of wild mushroom extracts of *R. delica* and

L. giganteus against *E. coli.* The extract of *L. giganteus* showed better synergistic activity against ESBL producing *E.coli* with ampicillin, ciprofloxacin and trimethoprim / sulfamethoxazole. Antimicrobial activity of medicinal mushroom *Ganoderma lucidium* was investigated in combination with four kinds of antibiotics ampicillin, cefazolin, oxytetracycline and chloramphenicol and the fractional inhibitory concentration index revealed additive effect in most instances, synergism and antagonism in two cases individually. Synergism was obtained when extract of *Ganoderma lucidium* was combined with cefazolin against *Bacillus subtilis* and *Klebsiella oxytoca* [16]. This approach is beneficial as the bioactive components of the edible mushroom are expected not to show any toxic effects *in vivo*. This synergistic approach will be very much useful in future to lower the pace of drug resistance, as after the carbapenem group of antibiotics hardly any drug is available to treat the ESBL group of microorganisms [17]. Thus the unique feature of the present study is that we have showed here the synergistic effect of meropenem, conventionally known as last line of drugs in combination with edible mushroom extracts to act against multidrug resistant bacteria.

5. CONCLUSION

So far, there is no study regarding the synergistic effect of edible mushrooms extracts with conventional antibiotic against multidrug resistant microorganisms. So the positive outcome of this study may significantly help in fighting against multidrug resistant bugs in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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