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Original Article

Microbiology Section

Evaluation of Antibacterial Activity of Exogenous Glutathione and its Synergism with Meropenem against Clinical Isolates of *Acinetobacter baumannii*

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ABSTRACT

Introduction: *Acinetobacter baumannii (A. baumannii)* is the major nosocomial pathogen especially associated with ventilator associated pneumonia. It often poses significant challenge in treatment as it is resistant to almost all classes of antibiotics. Glutathione (GSH), a tripeptide antioxidant has been demonstrated with antibacterial activity in various bacteria.

Aim: To detect the antibacterial activity of exogenous GSH against clinical isolates of *A. baumannii* and its synergism with meropenem.

Materials and Methods: This prospective cross- sectional study was conducted at Department of Microbiology, Pondicherry Institute of Medical sciences, Puducherry, India. Sixty clinical isolates of *A. baumannii* from various clinical specimens between August to September 2018 were included in the study. The antibacterial activity of GSH at concentrations 10-20 mmol/L and meropenem were determined by broth dilution method. To determine the synergism, onto Muller Hinton Agar (MHA) with GSH (at Minimum Inhibitory Concentration (MIC)), Epsilometer (E) strips containing meropenem were placed over the lawn cultures. Fractional Inhibitory Concentration (FIC) of the isolates was also determined to demonstrate synergism of GSH with meropenem. The results were analysed by percentages and proportions.

Results: Levels of MIC ranged from 16-18 mmol/L of exogenous GSH and >32 µg/mL for meropenem. The MIC of meropenem in the presence of GSH for all the isolates were <0.25 µg and the FIC <0.5, suggesting GSH synergistically interacts with meropenem.

Conclusion: The GSH showed antibacterial activity against *Acinetobacter baumannii* and also acted synergistically with meropenem to reduce its MIC. Hence this study demonstrates the potential therapeutic utility of exogenous GSH against *A. baumannii*.

Keywords: Antioxidant, Carbapenem, Ventilator associated pneumonia

INTRODUCTION

Antimicrobial resistance is a serious global threat with the emergence of multidrug resistance organisms. Misuse and overuse of antibiotics has provided strong selective pressure on microorganisms, leading to preferential survival and spread of those with antibiotic resistance mechanisms. From multidrug resistance, bacterial pathogens have developed further to antibiotic resistance now affecting all antibiotic classes [1]. This is particularly worrisome in the case of gram negative bacteria especially *Acinetobacter baumannii*.

Acinetobacter baumannii, a gram negative pathogen is ubiquitous in nature and has been recovered from soil, water, animals and humans. The infections due to *A. baumannii* has high incidence among immunocompromised individuals particularly those who have experienced prolonged hospital stay. The major clinical impact of *A. baumannii* is nosocomial infection including ventilator associated pneumonia [2]. It is nowadays emerging as cause of numerous global outbreaks as well as endemic strain in Intensive Care Units (ICUs). Its propensity to cause outbreaks is related to its multidrug resistance and its resistance to desiccation [3]. The treatment of this pathogen is almost difficult because of its innate and acquired resistance to almost all commercially available antibiotics [4]. Hence, there is an urgent need to develop newer therapeutic options for treating this pathogen.

The GSH, a tripeptide, is one of the most important intra and extracellular antioxidants, providing protection against oxidative stress. It participates in detoxification of xenobiotics, regulation of cellular growth, modulation of immune response and maintenance of the thiol status of proteins and cellular cysteine levels [5]. GSH is also known to have a regulatory effect on immune cells and even inherent antibacterial properties have been reported [6]. Though its antibiotic properties have also been evaluated against various organisms like *Pseudomonas* and *Staphylococcus*, the clinical relevance and the molecular details are unclear [6]. GSH scavenges and inactivates Reactive Oxygen Species (ROS), thereby reducing their cytotoxicity. When cells are exposed to oxidant species, GSH is able to reduce ROS and itself is oxidised into glutathione disulphide (GSSG). GSSG is reduced back to GSH through the action of GSH reductase. This cycling of GSH is an important means of limiting oxidative damage in the cells [7].

Exogenous GSH either alone or in combination with current antibiotics is also applicable in treating infections caused by multidrug resistant *Pseudomonas aeruginosa* [8]. GSH is also reported to have antibiofilm property against *Pseudomonas aeruginosa* and acts as a potential biofilm disrupter agent when combined with antibiotics like ciprofloxacin or tobramycin [9]. It has also been found to modify the sensitivity of resistant strains of *Staphylococcus aureus* and thereby improves the bactericidal action of ciprofloxacin and gentamicin against *S. aureus* [10]. GSH also shown to enhance the antibacterial activity of β-lactam antibiotics against *Escherichia coli* [11], but there are few literatures on the antibacterial activity of GSH in *A. baumannii* [12-14]. The current study was undertaken with the objective: i) To detect the antibacterial activity of exogenous GSH against clinical isolates of *A. baumannii* and to determine its MIC; ii) To determine the synergism of GSH with meropenem.

MATERIALS AND METHODS

The present prospective cross-sectional study was conducted in the Department of Microbiology at Pondicherry, India Institute of Medical Sciences, Puducherry between August to September 2018

for duration of two months. The study was approved by the Institute Ethics Committee (RC/18/15) and a waiver of consent was obtained as the study did not involve human participants.

Inclusion criteria: During this period about 60 isolates of *Acinetobacter baumannii* from various specimens like blood, endotracheal aspirates, wound infection etc. were included in the study by convenient sampling. These isolates were stored at 4ºC until further testing.

Exclusion criteria: Repeat isolates from the same patient were excluded from the study.

Determination of Antibacterial Activity of GSH [12]

Antibacterial activity of GSH (Himedia) was measured by MIC and Minimum Bactericidal Concentration (MBC) for all clinical isolates. Appropriate concentration of GSH (10-20 mmol) was prepared in 1 mL of Mueller Hinton (MH) broth. Fresh overnight culture of each isolate of *A.baumannii* was diluted in saline (~10^8 cells/mL) and inoculated in the MH broth supplemented with different concentrations of GSH. The inoculated cells were incubated for 18 hour at 37°C. From this MIC level was determined as the lowest concentration of GSH that completely inhibited the growth. MBC levels were determined by the cellular growth of inoculums used for MIC measurement on MacConkey agar plates. Concentration of GSH which shows 100% inhibition of cellular growth was taken as MBC.

Determination of Minimum Inhibitory Concentration (MIC) of Cultured Isolates to Meropenem

MIC of each isolate to meropenem was determined by E test (Himedia) as per CLSI 2019 [13]. Concentration of the meropenem at which the zone intersects the E strip was taken as the MIC. MIC levels <2 µg/mL was taken as sensitive and >8 µg/mL was taken as resistant.

Determination of Synergism of GSH with Meropenem

To determine the synergism, MHA with GSH (at MIC) was prepared. To the MHA with GSH, lawn culture of the isolates were prepared and E strips containing meropenem were placed. The MIC of meropenem in the presence of GSH was interpreted by the same procedure as mentioned above. FIC was calculated using the following formula:

FIC index of glutathione=Lowest MIC of GSH in combination with antibiotic/MIC of GSH alone. FIC index ≤0.5 was taken as synergism.

STATISTICAL ANALYSIS

Statistical analysis was carried out by percentages and proportions.

RESULTS

About 60 isolates of *Acinetobacter baumanni* isolated from various clinical specimens were included in the study. The majority of the isolates were from endotracheal aspirates (55%) obtained from patients on ventilation [Table/Fig-1]. All the isolates (100%) were resistant to all class of antibiotics tested i.e., cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.

Specimen	No of Isolates (%)
Endotracheal aspirate	33 (55)
Wound swab	18 (30)
Tissue	3 (5)
Blood	3 (5)
Sputum	1 (1.7)
Bronchoalveolar lavage	1 (1.7)
Pleural fluid	1 (1.7)

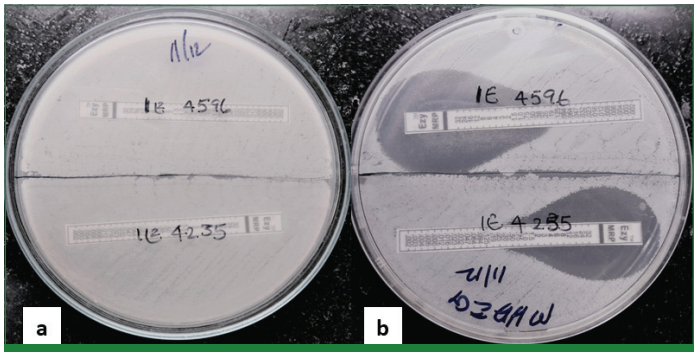
[Table/Fig-1]: Distribution of clinical isolates.

The antibacterial activity of GSH was tested at various concentrations (10-20 mmol/L) and is shown in [Table/Fig-2]. Further the cellular growth of these bacteria was completely inhibited at 19 mmol/L.

MIC of Glutathione (mmol/L)	No of Isolates (%) n=60
16	6 (10)
17	12 (20)
18	42 (70)

[Table/Fig-2]: Antibacterial activity of Glutathione (GSH).

The MIC of all these isolates when tested to meropenem was >32 µg/mL making them resistant to it. When tested in the presence of GSH, MIC of these isolates reduced to 0.25 µg/mL making them susceptible to meropenem [Table/Fig-3]. FIC index of all the isolates were ≤0.5 suggesting that GSH exhibits synergism with meropenem [Table/Fig-4].



[Table/Fig-3]: Showing MIC of Meropenem >32 µg (a) and the same isolates showing MIC of Meropenem of 0.2 µg in the presence of glutathione (b).

Antibiotic MIC (µg/mL)	No of isolates	FIC index
Meropenem (>32)	60	
Meropenem+Glutathione (0.25)	48	0.0078
Meropenem+Glutathione (0.38)	7	0.0119
Meropenem+Glutathione (0.5)	5	0.1563

[Table/Fig-4]: Synergism of GSH (18 mmol/L) with meropenem.

DISCUSSION

The antibiotic resistance in *A. baumannii* infection has become a global crisis. The fast development of antibiotic resistance in *A. baumannii* infection warns of an absolute "post-antibiotic era" for these infections, if resistance to tigecycline and colistin become common in the next 10-20 years [8]. Hence, there is a need for development of new antibiotic to treat these infections. Earlier studies have shown that GSH is a potent adjuvant with antibacterial activity demonstrated against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E.coli* [9-11].

Antibacterial Activity of GSH

Of the 60 clinical isolates of *Acinetobacter* studied, about 55% were from endotracheal aspirates and all these isolates were resistant to cephalosporins, aminoglycosides and carbapenams. The antibacterial activity of GSH tested by broth dilution method, showed a sharp decline in turbidity and hence MIC of 18 mmol/L (70%), 17 mmol/L (20%) and 16 mmol/L (10%). The MBC of all the isolates were 19 mmol/L. A similar study done on *Acinetobacter* by Alharbe R et al., showed that the levels of MIC and MBC ranged from 10-13 mM and 11-15 mM of GSH respectively [12]. These results were similar to previous studies on antibacterial activity of GSH done against *Pseudomonas* and *E. coli* [9,11]. Similar study done by Zhang Y and Duan K against *Pseudomonas* showed antibacterial activity by disk diffusion [7]. The role of GSH in bacteria is to maintain the optimum intracellular oxidation reduction potential which is required for the various physiological functions. Exogenous GSH damages the bacterial cell by producing ROS through hyperactivation of electron transport chain [14]. Other possible mechanisms as described by Kwon DH et al., is the import of exogenous GSH across the bacterial cell and disruption of homeostasis of GSH redox potential, thereby

altering the optimum pH levels [15]. In recent reports, GSH selenium nano-incorporation (GSH-SeN-Inco) have shown antimicrobial activity against gram-negative especially *Escherichia coli* ATCC 25922, *Campylobacter* spp [16,17].

Synergism of Glutathione (GSH) with Meropenem

Carbapenems remain the mainstay for the treatment of *Acinetobacter* infections, though there is widespread emergence of resistance. Hence, meropenem was chosen for testing synergism with GSH. The results indicate that in the presence of GSH (18 mmol/L) the MIC of meropenam to most isolates reduced from >32 µg/mL to ≤0.25 µg/mL. The data indicates synergism of meropenam and GSH with FIC index of all the isolates ≤0.5. Alharbe R et al., had shown similar results that subinhibitory concentration of GSH (6 mmol/L) synergistically killed carbapenem associated multidrug resistant isolates in combination with meropenem or other conventional antibiotics [12]. It is hypothetised that exogenous GSH is associated with inhibition of multidrug efflux pumps and/or down-regulation of antibiotic resistant genes, thereby explaining the synergism between meropenem and GSH [18,19].

The findings from this study suggested that GSH is a therapeutic option that can be used against carbapenem resistant *Acinetobacter baumannii* isolates. Though inhalational GSH is used as therapeutic option to treat cystic fibrosis, its potential as antibacterial agent at concentrations used in our study is not known [20]. Hence, further studies in animal models are recommended to detect the possible cellular toxicity at these concentrations. Nevertheless, GSH can be used in vitro as incorporation in endotracheal tubes or catheters thereby preventing the colonisation and hence nosocomial infections caused by this pathogen.

Limitation(s)

Small sample size due to the limited study period. Due to limited resources, standard method like checker board assay was not done to demonstrate synergism.

CONCLUSION(S)

This study demonstrates the antibacterial activity of GSH and its therapeutic potential against carbapenam resistant *Acinetobacter baumannii*. Moreover, it also enhances the antibacterial effect of meropenem to these clinical isolates. However, further studies in animal models are recommended to study the safety and efficacy of GSH at the recommended doses.

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