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Research Article

IN VITRO MEROPENEM SUSCEPTIBILITY INDUCED BY PH ALTERATION IN METALLO-BETA-LACTAMASE POSITIVE *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objective: The increasing emergence of metallo-beta-lactamase (MβL) producing Gram-negative bacteria such as *Pseudomonas aeruginosa* pose a serious public health concern including in the treatment of urinary tract infection (UTI). This study was aimed to explore the combined effect of *in vitro* pH alteration and antibiotic on the bacterial growth as a potential therapeutic approach against the drug resistant *P. aeruginosa.*

Methods: Ten MβL producing *P. aeruginosa* isolates from the patients suffering from UTI were included. Bacteria were inoculated with or without meropenem in media with varied pH range from 5 to 10. The variation of bacterial growth was determined by measuring the changes of optical density at 620 nm and colony forming unit counts/ml.

Result: The growth of bacteria was reduced both at very high and lower pH ranges. However, the growth was further reduced significantly with the addition of meropenem at these extreme pH conditions.

Conclusion: Alteration of pH especially at lower range of the medium might has changed the efficacy of MβL and thus helped the antibiotic meropenem to act on the bacteria, which was resistant toward the same at neutral pH. Combined effect of antibiotic and pH modulation of biological fluid like urine should be explored for an effective alternative therapeutic approach against the drug-resistant bacteria.

Keywords: Metallo-beta-lactamase, urinary tract infection, *Pseudomonas aeruginosa*, pH.

INTRODUCTION

The development of drug resistance among the common pathogens till now is the most serious and difficult problem to deal with [1]. The resource of antimicrobial agents is limiting and demand of alternative chemotherapeutic approaches are increasing.

Multi drug resistance (MDR) is a common scenario in hospital acquired infections. *Pseudomonas aeruginosa* accounts for about 10% of all hospital acquired infections [2,3]. Moreover, *P. aeruginosa* is one of the important Gram-negative organisms responsible for severe urinary tract infection (UTI) and associated with significant morbidity. The bacteria can invade the bloodstream from UT and is the source of nearly 40% nosocomial infections due to catheter-associated UTI leading to serious complications [4]. Carbapenem resistance which is acquired metallo-beta-lactamase (MβL) and reported mainly due to *P. aeruginosa* from various countries [5]. Moreover, resistance toward colistin has also emerged several years back making the scenario more complicated. MβL producing *P. aeruginosa* being significantly MDR is a very difficult to treat [6].

Various approaches have been made to combat the MDR pathogens including combined chemotherapies, chemical/herbal extracts, and combination of both [7]. Still these methods have limitations and require more avenues to be opened. Based on the fact that pH has great effect on the enzymatic activity and bacterial growth, it has been hypothesized that *in vitro* pH alteration might has effect on the bacterial growth as well as the MβL activity. The pH optima of MβL are around 7.4 [8]. In this study, we highlight an alternative chemotherapeutic approach (*in vitro*) to control the MβL producing *P. aeruginosa* by modifying of pH of the growth medium and addition of a potent β-lactam antibiotic meropenem.

METHODS

Bacteria

To investigate the fact, a total of ten (n=10) clinical isolates of MβL producing *P. aeruginosa* were included. The strains were previously isolated from the patients suffering with UTI and maintained at our hospital.

The antimicrobial susceptibility of the isolates was tested according to the Clinical and Laboratory Standards Institute guideline [9], and all were resistant to third generation cephalosporins. Briefly, the MβL screening was done by imipenem disc diffusion test followed by imipenem-EDTA combined disc and imipenem-ethylenediaminetetraacetic acid (EDTA) synergy tests [10].

Effect of pH on bacterial growth

Bacteria were inoculated in Mueller-Hinton broth (HiMedia) with varying pH ranges from 5 to 10. In one set of experiment, bacteria were incubated with meropenem (Astra Zeneca, UK) (16 µg/ml) and in another set no antibiotic was added in 96 well-microtiter plate. After overnight incubation at 37°C, the variation of bacterial growth was determined by measuring the changes of optical density at 620 nm followed by colony forming unit count/ml.

Statistical analysis

Each experiment was repeated thrice and mean values were taken for analysis. Finally, the data are represented as mean ± standard deviation and analyzed using Graph Pad Prism (version 5). $p<0.05$ was considered as significant.

RESULTS

The results indicate the significant differences between the bacterial growth with and without the addition of meropenem both at the

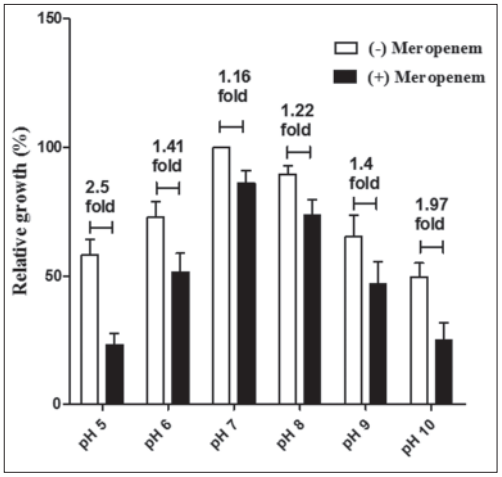

Fig. 1: Bar diagram represents the relative growth of *Pseudomonas aeruginosa* (n=10) at various pH ranges (pH 5-10) with reference to the growth at pH 7 without meropenem (16 µg/ml). The data are represented as mean±standard deviation. Fold decrease of growth are shown in the figure

alkaline as well as acidic pH ranges. However, the relative growth was least at the pH 5 (2.5 fold) compared to control without the antibiotic at the same pH (Fig. 1). Moreover, at the higher pH ranges, i.e., pH ≥9, the growth of the bacteria were markedly decreased. Overall, the data indicate that at very low and high pH ranges (pH ~5 and pH~10); the bacterial growth is significantly reduced in the presence of meropenem (16 µg/mL) ($p<0.0001$ by two-way ANOVA).

DISCUSSION

To test our hypothesis, we incubated the organism at various pH ranges to measure the effect of pH only and also with the addition of antibiotic to measure the efficacy of the enzyme MβL. From the result, it is clear that alteration of pH has significant effect on bacterial growth *in vitro*. Interestingly, the growth was further retarded with the addition of antibiotic to which the bacteria were resistant at neutral pH. At the very higher and lower pH ranges, the relative growth was significantly retarded. The reason might be that at the lower and higher pH ranges, the efficacy of the enzyme MβL have reduced and could not act on meropenem properly, helping the antibiotic to act on the organisms easily. This concept is also supported by previous studies demonstrating the effect of pH modulation on the alteration of antimicrobial susceptibility pattern of various organisms [11,12]. Corroborating to the present *in vitro* finding and former research works, it can be proposed that pH alteration of the biological fluids like urine can alter the meropenem resistance of the organism for better control of the pathogen. An average pH of urine is usually 6.5 [13] and this can vary according to the food intake. Overall, it has been found that acidic pH was more effective over the alkaline pH in lowering the growth of this uropathogenic bacteria.

CONCLUSION

Lowering the pH of urine could be achieved easily by administration of various agents like Vitamin C [14]. Hence, Vitamin C in combination with meropenem could be an effective chemotherapeutic approach to combat the MβL producing Gram-negative bacteria in urine. This *in vitro* study may reflect the situation *in vivo* and contribute in developing alternative antimicrobial strategy against MDR Gram-negative organisms. However, the extrapolation of the data should be done cautiously.

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