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Original Research Article

Studies on different antibiotic principles against methicillin, vancomycin and linezolid resistant strains

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A B S T R A C T

The treatment of bacterial infections urgently requires alternative approaches because the use of antibiotics is becoming increasingly restricted due to the emergence of antibiotic resistant mutants. The MIC values of the different antibiotics rifampicin, isoniazid, rifabutin, streptomycin, spectinomycin, levofloxacin and linezolid were determined against multidrug resistant strains like MRSA, VRSA, linezolid resistant strains *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230 by the serial dilution method. The activity of the rifampicin, isoniazid and rifabutin against MRSA, VRSA and other *E. faecium* species was more effective with low MIC values than existing drugs like vancomycin and linezolid. These drugs may be the potential alternative drug therapy for multidrug-resistant organisms and warrants further investigation.

Introduction

Alternative approaches in the treatment of bacterial infections are urgently required because the use of antibiotics is becoming increasingly restricted due to the emergence of antibiotic resistant mutants (Dharmaratne *et al.,* 2013). The antibiotic research from the discovery of Flemming to our days has been a fascinating, exciting, continuous changing and developing adventure (Berdy, 2005).

Acquiring resistance to a specific antibiotic provides a clear benefit to the bacterium when exposed to that antibiotic. Thus, the acquisition of antibiotic resistance was commonly cited as an example of "evolutionary change". Bacteria and other micro organisms that cause infections are remarkably resilient and can develop ways to survive drugs meant to kill or weaken them. This is called as antibiotic resistance, also known as antimicrobial resistance or drug resistance (Anderson, 2005). Drug resistance presents an ever-increasing global public health threat that involves all major microbial pathogens and anti-microbial drugs. The real impact of resistance is manifested in the hospital environment, where the major kinds and numbers of resistant pathogens have

increased over the past decade. Overall, in the United States and United Kingdom, 40–60% of nosocomial *S. aureus* strains are methicillin-resistant (MRSA) and usually MDR. More deaths are associated with MRSA than methicillin-sensitive strains. A steadily increasing, small proportion of MRSA also showed low-level resistance to vancomycin (Levy and Marshall, 2004). However, as with every other great discovery that revolutionized human life, new problems had arisen as a result of the usage of antibiotics. The prevalence of certain microbes resistant to specific antibiotics was now a major problem of chemotherapy. As resistant forms appear, new antibiotics, or new forms of known antibiotics, have to be found to eradicate them. This seemed to be an endless process (Waksman and Lechevalier, 1962). New compounds in the β-lactam, macrolide, tetracycline and quinolone families were produced that have advantages in spectrum, potency and/or pharmacology, such as improved oral absorption or longer half-life (Neu, 1983).

Strategies to overcome the problem of antibiotic resistance

The development and use of new antimicrobial agents is the most obvious way to combat the emergence of antimicrobial resistance. However, extending the life of current antibiotics could be achieved by more appropriate use of existing antimicrobial agents. Less use of antimicrobial agents would lead to a reduction in resistance and an extension to the effective life of these agents (Carbon and Bax, 1998).

Chemical synthesis can be used to make fundamentally new structures that might act at different bacterial targets to those already identified. Drugs arising from such approaches include the oxazolidinones and ketolides (Bax and Mullan, 1999).

In view of this, to find potential antibiotic producers capable of acting on clinical resistant strains, we have chosen some antibiotics like rifampicin, isoniazid, rifabutin, streptomycin, spectinomycin, levofloxacin and linezolid and tested against clinical and resistant pathogens like linezolid resistant *Enterococcus faecium* (JM-H, SM-T & CC-X), Vancomycin resistant *Staphylococcus aureus* (VRSA) and Methicillin resistant *Staphylococcus aureus* (MRSA) by serial dilution method.

Materials and Methods

Multidrug resistant microorganisms

The multi drug resistant strains – Linezolid resistant *Enterococcus faecium* (JM-H (5684), SM-T (23230) & CC-X (48585)) were obtained from New York medical centre, New York. Vancomycin resistant *Staphylococcus aureus* (VRSA) (ATCC 43300) from Christian Medical College, Vellore and Methicillin resistant *Staphylococcus aureus* (MRSA) (S 101) were obtained from Clinical lab of MGM Hospital, Warangal, A.P, India. Antibiotics Rifampicin, Isoniazid, Rifabutin and Levofloxacin were generous gift samples from Lupin Pharmaceuticals, Mumbai, India. Spectinomycin was obtained from Cipla ltd., Goa. Linezolid was gift sample from Piramal health care, Mumbai. Streptomycin sulfate (Ambistryn-S) was purchased from Sarabhai Piramal Pharmaceutical Private Limited, Vadodara. All other polymers and solvents used were of analytical grade.

Preparation of slants

Rimless test tubes of 15 cm in length were filled with 5 ml of respective medium, plugged with cotton and sterilized in the autoclave. When the sterile medium was in

warm molten state, the tubes were kept in slanting position.

Preparation of agar plates

Sterile Petri plates of 9 cm in diameter were filled aseptically with 27 ml of warm sterile liquefied agar medium in the laminar air flow bench. The plates were incubated in an inverted condition at 37^{o} C for one day to check for contamination of bacteria.

Preparation of antibiotic stock solutions

Antibiotic concentrations of rifampicin, isoniazid, rifabutin, streptomycin, spectinomycin, levofloxacin and linezolid were prepared in the range of 0.2–100 µg/ml.

Preparation of inoculum

The inoculum should be adjusted so that 104 cfu/spot are applied to the plates. The following procedure describes a method for preparing the desired inoculum by comparison with a 0.5 McFarland standard.

Preparation of the McFarland standard

0.5 mL of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2 \cdot 2H_2O$) was added to 99.5 mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring. The standard was distributed into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. The tubes were sealed tightly to prevent loss by evaporation and protected from light at room temperature. The turbidity standard on a vortex mixer was vigorously agitated before use. Standards may be stored for up to 6 months, after which they should be discarded. Alternatively, prepared standards can be purchased (bioérieux, Basingstoke, UK).

Determination of minimum inhibitory concentration (MIC) of antibiotics by serial dilution method

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints. MBC determinations are undertaken less frequently and their major use has been reserved for isolates from the blood of patients with endocarditic.

The *in vitro* antibacterial assay of the antibiotics (rifampicin, isoniazid, rifabutin, streptomycin, spectinomycin, levofloxacin and linezolid) were performed by the serial dilution method to find out minimum inhibitory concentration (Andrews, 2001) in Muller Hinton broth, at different concentrations, viz., 0.2–100 µg/mL by employing 24 h cultures of vancomycin resistant *Staphylococcus aureus* ATCC 43300, methicillin resistant *Staphylococcus aureus* S 101, *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230.

The inoculum was adjusted to Mc Farland standard and added into the medium.

Transfer the inoculated broth into labeled test tubes.

Each compound was tested in triplicate.

Serial dilution method

Required quantity of Muller-Hinton broth was prepared and sterilized by using autoclave. Test tubes also sterilized in autoclave with a tight cotton plug and labeled from 100µg/ml–0.2µg/ml. After sterilization the prepared Muller-Hinton broth (1ml) was taken into each test tube. The antibiotic solution of 1 ml containing 200µg/ml was added to the first test tube labeled as 100µg/ml. One ml solution from the first tube was transferred to 2^{nd} tube which is labeled as 50µg/ml. From the 2^{nd} test tube 1ml of the solution is taken and transferred into another test tube which is labeled as 25µg/ml. In this manner dilutions were carried out for the remaining test tubes. After the completion of serial dilution method the test tubes were kept in incubator for overnight.

Incubation conditions

Conditions for incubation are 37± 2°C for 24 h.

Reading and interpretation

The MIC for the control strain should be within plus or minus one two-fold dilution of the expected MIC. Results were observed after 24 h of incubation. MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity.

Results and Discussion

Determination of Minimum inhibitory concentration (MIC) of antibiotics against clinical resistant strains

The MIC values of the different antibiotics (rifampicin, isoniazid, rifabutin, streptomycin, spectinomycin, levofloxacin and linezolid) were determined for the multidrug resistant strains by the serial dilution method and the results are depicted in Table 1. The lowest values were recorded for the antibiotics rifampicin, isoniazid, rifabutin, levofloxacin and linezolid.

The MIC value of the rifampicin against MRSA, VRSA, linezolid resistant strains *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230 was found to be 0.8, 0.8, 0.4, 3.12 and 0.8 µg/ml respectively. The activity of the rifampicin against MRSA, VRSA and other *E. faecium* species was more effective than existing drugs like vancomycin and linezolid. The MIC of linezolid against MRSA was found to be 28 µg/ml, where as for vancomycin was at 30 µg/ml (Kaleem *et al.,* 2011). The MIC of linezolid against VRSA was found to be 4 µg/ml. So this can be a lead molecule for further development in the multidrug resistant therapy.

The MIC value of the isoniazid against MRSA, VRSA, linezolid resistant strains *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230 was found to be 1.6, 1.6, 6.25, 6.25 and 3.12 µg/ml, respectively. The activity of the isoniazid against MRSA, VRSA and other *E. faecium* species was more effective than existing drugs. So this can be an alternative for the existing drugs.

The MIC value of the rifabutin against MRSA, VRSA, linezolid resistant strains *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230 was found to be 0.8, 0.8, 0.4, 3.12 and 1.6 µg/ml respectively. The MIC's of rifabutin against VRSA and *E. faecium* SM-T are very low and interesting compared with existing drugs like vancomycin and linezolid. rifabutin showed excellent activity against multidrug resistant strains.

The MIC value of the levofloxacin against

MRSA, VRSA, linezolid resistant strains *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230 was found to be 1.6, 0.4, 1.6, 1.6 and 0.8 µg/ml, respectively. The activity of the levofloxacin against MRSA, VRSA and other *E. faecium* species was more effective than existing therapy. It showed very good activity against VRSA.

The MIC value of the linezolid against MRSA, VRSA, linezolid resistant strains *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230 was found to be 25, 3.12, No activity, 100 and 50 µg/ml respectively. The activity of the linezolid against VRSA was more effective than MRSA and *E. faecium* species.

In this work, systematic efforts were made to find out the minimum inhibitory concentrations (MIC's) of some of the antibiotics against multidrug resistant strains. From the above results we can conclude that rifampicin, rifabutin, isoniazid, levofloxacin and linezolid showed lowest minimum inhibitory concentrations against multidrug resistant strains like vancomycin resistant *Staphylococcus aureus* ATCC 43300, methicillin resistant *Staphylococcus aureus* S 101, *E. faecium* CC-X 48585, *E. faecium* JM-H 5684 and *E. faecium* SM-T 23230.

These activities in tandem will be very interesting for further drug development of rifampicin, rifabutin and isoniazid in treating multidrug resistant infections.

Table.1 MIC's of antibiotics against multidrug resistant strains

S.No	Drug	Methicillin Resistant *Staphylococcus aureus* (MRSA) (ATCC 43300)	Vancomycin Resistant *Staphylococcus aureus* (VRSA) (S 101)	*E. faecium* *CC-X* 48585	*E. faecium* *JM-H* 5684	*E. faecium* *SM-T* 23230
		Minimum Inhibitory Concentration (MIC) (µg/ml)				
1	Rifampicin	0.8	0.8	0.4	3.12	0.8
2	Isoniazid	1.6	1.6	6.25	6.25	3.12
3	Rifabutin	0.8	0.8	0.4	3.12	1.6
4	Levofloxacin	1.6	0.4	1.6	1.6	0.8
5	Spectinomycin	No activity	100	No activity	No activity	No activity
6	Streptomycin	50	50	100	No activity	12.5
7	Linezolid	25µg/ml	3.12	No activity	100	50

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