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SYNTHESIS OF SILVER NANOPARTICLE ($AgNo_3$) FROM MARINE BROWN ALGAE *SARGASSUM MUTICUM* AGAINST BLOOD STREAM INFECTION CAUSING GREATEST WORLD KILLER PSEUDOMONAS AERUGINOSA

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ABSTRACT

Recurrently blood stream infection causing *Pseudomonas aeruginosa* is frequent pathogen associated with hospital acquired infections exhibiting intrinsic resistance to numerous antibiotics. A total of 50 isolates of *Pseudomonas aeruginosa* were isolated from blood cancer patient from primary healthcare center around Tirupur District and the isolates were identified using phenotypic characterization. Further, the microbial sensitivity testing was done using Disk Diffusion Test (DDT). In this study *Pseudomonas aeruginosa* demonstrated marked resistant against different class of antibiotics. Among different class of antibiotics the maximum resistant activity was observed in Vancomycin 99% followed by minimum resistant was observed in Levofloxacin 1%. This result proved the greatest world killer (*Pseudomonas aeruginosa*) is responsible for the demise of cancer patient annually. It indicated that the impact of blood stream infection causing *Pseudomonas aeruginosa* is greater significance in developing countries. So for the management of disease is troubled by simultaneous resistance to multiple drug representing that there is severe and pressing need to progress new therapeutics that can alarmed drug resistance. Recurrently, the marine brown algae has been considered for varied and plentiful source of potent bioactive chemical such as sliver nanoparticle ($AgNo_3$) from *Bacillus cereus* which can be represented as drug of choice. The nanoparticles showed appreciable activity at all tested concentrations (50, 100 and 150µl). Thus, it is concluded that the present study designed to determine the synthesis of sliver nanoparticle ($AgNo_3$) from brown algae which serve as a promising antibacterial agent against BSI.

KEYWORDS: Blood stream infection, *Pseudomonas aeruginosa*, Brown algae, Sliver nanoparticle ($AgNo_3$).

INTRODUCTION

According to the World Health Organization, Blood stream infection (BSI) is a leading cause of death worldwide, with approximately 14 million new cases annually.[1]

Recurrently, bacterial bloodstream infections are serious infections associated with significant mortality and health care costs.[2] In many hospitals, Pseudomonas aeruginosa has become the most common gram negative bacterial species associated with serious hospital acquired infections, particularly within intensive care units.[3]

Infectious diseases can be classified as emerging and re-emerging infectious diseases. The diseases which are new are referred to as emerging infectious diseases, whereas re-emerging infectious diseases refer to infections which are not new, but suffer from drug-resistance when they reappear, thereby making them difficult to treat or control.[4] Although the human immune system has the ability to defend the body from infections, some infections however are easily transmitted, while others are very contagious and virulent in nature.[5] Because, today the wide spread emergence of multidrug resistance among bacterial pathogens has become one of the most serious challenge in clinical therapy.

On the other hand, some of infections are not contagious. Overall, infections can be transmitted when microorganisms causing infections enter the host body via natural orifices resulting in the microorganism growing at the site of entry followed by multiplication in

the host cells resulting in tissue damage.[5] However, it is important to mention that some microorganisms can replicate in the extracellular spaces within the body resulting in tissue damage. The management of communicable diseases is stalled by drug resistance, demonstrating that there is a severe need to grow new therapeutics which can overcome drug conflict.

Recurrently, biosynthesis of nanoparticles is the emerging field in nanoscience and nanotechnology. Using of biological entities such as brown algae associated bacteria for the synthesis of silver nanoparticles have been developed. Among varied nanoparticle sliver nanoparticles are increasingly used in various fields including pharmaceutical and industrial purpose and also due to their peculiar properties, they have been used for several application including as medical device coding, optical sensors, as cancer agents, in diagnostic, drug delivery and have ultimately enhanced the tumor killing effects of anticancer drugs.[6] So hence the present has made an attempt to point out the comprehensively addressed synthesis, of silver nanoparticles (AgNo3) from seaweed brown algae *Sargassum muticum* against blood stream infection causing *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Sample collection

Totally fifty cancer blood samples were collected from different clinics in rural area of Tirupur, Tamilnadu, in the period of March to April 2019 using transport media. It was place in ice pack box and were brought to Division of Microbial Technology, PG and Research Department of Zoology, Chikkanna Govt. Arts College, Tirupur-641602. After reached to the laboratory the samples were incubated at 37ºC for 24 hours. Finally the completion of the incubation period the samples were used for isolation of BSI pathogen.

Isolation of *Pseudomonas aeruginosa* from cancer blood sample

All the strains isolated from blood stream (cancer) infected samples were serially diluted from 10^{-1} to 10^{-9} and the dilutions 10^{-4} to 10^{-6} were plated onto Cetrimide agar it was incubated at 37°C for 48hours. The individual colonies with different morphology were picked using sterile tooth pick and grown in nutrient broth and it was incubated at 37°C for 24hours. Further it was plated to check for purity.

Identification of BSI pathogen *Pseudomonas aeruginosa*

In this study isolates were identified using biochemical characterization according to Bergy's Manual of Systematic Bacteriology.

Antibiotic susceptibility testing by disc diffusion test

Antibiotic susceptibility was semi-quantitatively determined by disc diffusion method. The culture was inoculated into the Muller Hinton broth which was incubated at 37°C for 12hours. Plates were made with Mueller Hinton agar and allowed to solidified 10 to 15 minutes. Then 0.1ml of this culture were inoculated in the plates using L-Rod by spread plate technique. The antibiotic disks: Kanamycin, Amikacin, Imipenam, Meropenam, Cephalexin, Erythromycin, Vancomycin, Carbenicillin, Tetracycline and Levofloxacin were placed in the plates. Agar plates with antibiotic disks were then incubated for 37°C 24 hours. The diameters of the inhibition zone were measured using a ruler under a colony counter apparatus. The results were expressed as sensitive (S), marginally susceptible (I), and resistant (R).

Collection of marine brown algae

Seaweeds were collected by hands picking in period of May – June, 2019 from the Islands for the first time from Thoppu Kaadu theevu, Mandapam along the Gulf of Mannar Coast [8.47°N 79.02°E], Rameswaram, Tamilnadu, India. During the collection, Brown algae *Sargassum muticum* were put into ziplock plastic bags and placed in a cool box. Then the samples were transported to the laboratory within 24 hrs after collection.

Laboratory Analysis

The each collected *Sargassum* species seaweeds were put into a sterile plate were initially, and the tissues of each *Sargassum* were rinsed with sterile seawater to remove the macroscopic epiphytes, debris, again washed with fresh water to remove the surface salts, sand particles and scraped off with a sterile knife. Then the tissue of sea weed was weighed in a sterile beaker, and ground in mortar and pestle. Then the sample was serially diluted (10^{-1} to 10^{-9}) and spread on to Zobell 2216E marine agar media. After the appropriate dilution 10^{-4} to 10^{-6} for the isolation of associated bacteria from the sample. The different dilutions were drawn and poured onto the surface of marine agar media for isolation. Finally, the plates were incubated at 37°C for 24 - 48 hrs.

Isolation and identification of bioactive potential bacteria associated with marine Sea weed *Sargassum Muticum*

In this study, the bio medically active *Bacillus cereus* was isolated from marine brown algae (Fig. 1) growing in marine agar media like MYP agar for synthesis of silver nanoparticle $AgNo_3$. Further the morphological and physiological characterization of the isolate was performed according to the methods described in Bergey's Manual of determinative bacteriology.

Fig. 1: Brown Algae - Sargassum Muticum.

Synthesis of sliver nanoparticle ($AgNo_3$)

In extracellular synthesis process, the culture supernatant of *Bacillus cereus* was used. The culture was grown in 250 ml conical flask containing 100 ml of sterile nutrient broth for 24 hours at 37 °C in rotary shaker at 220 rpm. After the incubation the culture was centrifuged at 10,000 rpm for 10 minutes and collects the supernatant. To this supernatant 1 mM of silver nitrate was added and change of colour was noted at 30 mins and 24 h of incubation. The change of colour was indicates the formation of silver nanoparticles and periodically analysed by Spectrophotometer at different wavelength. Further the nanoparticle was used against BSI pathogen (Fig. 2).

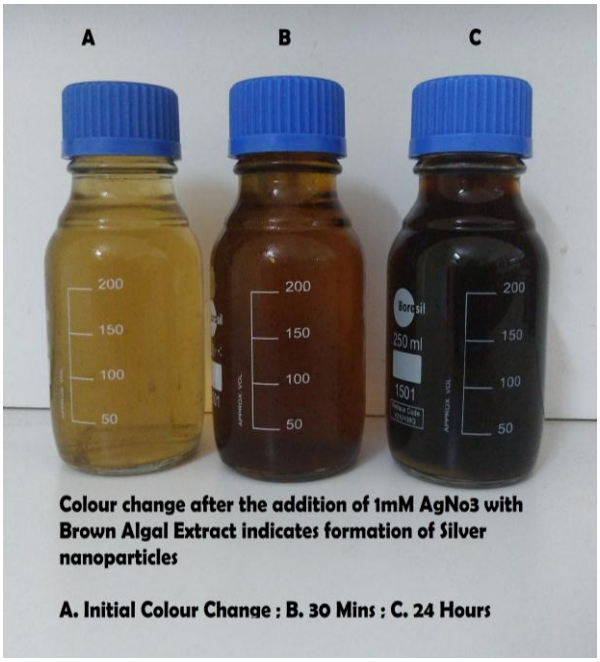

Fig. 2: Synthesis of silver nanoparticle (AgNo3).

Antibacterial activity of sliver nanoparticle ($AgNo_3$) from brown algae against blood stream infection causing *Pseudomonas aeruginosa*

The antibacterial activity of the $AgNo_3$ was performed by using well diffusion method. About 20 ml of sterile molten Mueller Hinton agar was poured into the sterile petri plates. Triplicate plates were swabbed with the overnight culture (108cells /ml) of pathogenic bacteria *Pseudomonas aeruginosa*. Different concentration of $AgNo_3$ (50µg, 100µg, 150µg) were prepared. The different concentration of sliver nanoparticle was screened against BSI pathogen. The isolates were selected on the basis of *Pseudomonas aeruginosa* should above 50% resistant against the antibiotics tested. The solid medium was gently punctured with the help of cork borer to make a well. Finally the $AgNo_3$ with the different concentration: 50µl, 100µl and 150µl were added from the stock into each well and incubated for 24h at 37±2°C. After 24 hrs of incubation, the zone of inhibition was measured and expressed as millimeter in diameter.

RESULT

Totally 50 blood cancer samples were collected from different age group of blood cancer patients in different clinics and PHC (Primary Health Centre) from in and around Tirupur District. 50 isolates of *Pseudomonas aeruginosa* were isolated from the samples. The *Pseudomonas aeruginosa* strains were confirmed by comparing the results with standard biochemical test of *Pseudomonas aeruginosa*. Selective media like Cetrimide agar media were used to isolate the carcinogenic pathogen. It showed green colour colony respectively. These colonies were isolated and stored for further experiment.

All the 50 carcinogenic *Pseudomonas aeruginosa* isolates were tested *invitro* to determine their antibiotic susceptibility patterns by antibiotic disc diffusion method. There are different group of 10 antibiotics isolates were assayed against 50 isolates of *Pseudomonas aeruginosa*. The diameter of the inhibition zone was measured using a ruler under a colony counter apparatus. The outcomes were expressed as sensitive, intermediate and resistant (R). The 50 isolates were exhibited the significance degree of resistant against Kanamycin (54%), Amikacin (63%), Imipenam (44%), Meropenam (56%), Cephalexin (59%), Erythromycin (84%), Vancomycin (99%), Carbencillin (60%), Tetracycline (70%) and Levofloxacin (1%). All the isolates showed multiple antibiotic resistance to the antibiotic tested. The maximum resistant was recorded in antibiotics such as Vancomycin 99% and maximum sensitive was observed in antibiotic such as Levofloxacin 1%.

Further, the after frequency analysis the Multiple Antibiotic Resistance (MAR) index was calculated according to the MAR index formula. Maximum MAR index 0.90 was showed by PAVG43 and minimum MAR index 0.55 was showed by PAVG07, PAVG09, PAVG14, PAVG26, PAVG27, PAVG34, PAVG37 and PAVG39.

The study proved the medical application of brown algae associated microbe producing nanoparticles is gaining popularity with an increasing number of nanoparticle based therapeutics currently in clinical development. We expect that with the introduction of safer nanomaterial together with novel engineering approaches that result in

optimally designed biologically synthesized nanoparticles like $AgNo_3$ may enter the clinic in future.

Further the antibacterial activity was tested using the different concentration of nanoparticle ($AgNo_3$) at different level such as 50µg, 100µg, and 150µg. In this study well diffusion assay was used. The result stated that the maximum activity was found in strain No. PAVG43 (17mm, 20mm and 25mm) and the minimum were recorded in PAVG07 (12mm, 19mm and 22mm) respectively (Fig. 3).

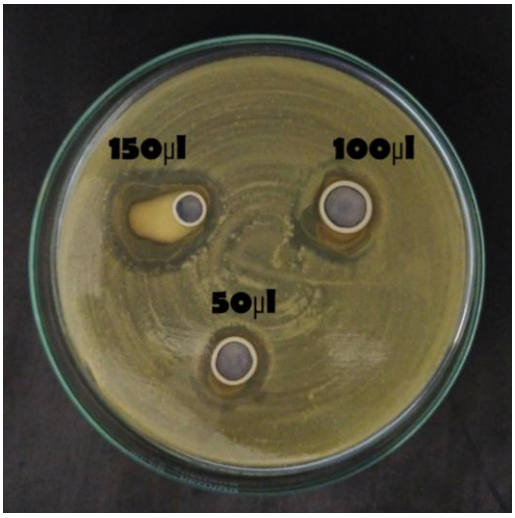

Fig. 3: Antibacterial activity of sliver nanoparticle from marine brown algae against blood stream pathogen Pseudomonas aeruginosa.

DISCUSSION

At presently, the emergence of resistant strains to commonly used antibiotics the microbial pathogens have necessitated the researchers to discover the new antimicrobial agents that are produced in natural way. The ocean, which cover almost 70% of the earth surface contain a variety species, many of which have no terrestrial counterparts [7]. Subsequently, in this study the associated marine microbe such as *Bacillus cereus* from brown algae may able to deliver the antimicrobial compound in the way of synthesize of nanoparticle AgNo3 for the treatment of blood stream infection causing greatest world killer pathogen *Pseudomonas aeruginosa.*

Resistance to imipenem has been found to be independent of β-lactamase production and in *P. aeruginosa* has been attributed to diminished expression of certain outer membrane proteins.[8] More than 80% of isolates in this study were sensitive to imipenem (66%). Compared with results of a study conducted at the Lagos University Teaching Hospital (LUTH) in which 12.5% were resistant to imipenem, but in this present study 44% *Pseudomonas* strains were resistant. Today the imipenem antibiotic is a drug that is not readily available in our environment and its cost is also prohibitive.

Analysis of the MAR index of the *P. aeruginosa* strains showed more than 50% of resistant had to be calculated maximum MAR index of 0.90, which is an indication of probable blood cancer origin from the hospital environment where antibiotics are extensively used. MAR index higher than 0.2 has been said to be an indication of isolates originating from an environment where antibiotics were often used [9]. The practical significance of the index may however be lost in Tirupur and Erode District where antibiotic use and abuse is rampant since the cutoff point was determined in countries with tight antibiotic control protocols. The MAR values can however be viewed as an indication of the extent of microbial exposure to antibiotics used within the community.

In this study, 63% of the *P. aeruginosa* strains were reportedly resistant to amikacin, in and around rural area of Tirupur Dt. While from the southern part of the country, less than 6.2% were resistant. Amikacin was the only antibiotic to which all the *Ps. aeruginosa* strains were susceptible. This is worrisome since amikacin is considered a potent agent in the treatment of infections caused by multiresistant *P. aeruginosa* and those strains that have shown resistance to gentamicin.[10]

Finally in this study we proved in fact our isolated *Pseudomonas aeruginosa* multidrug resistant from blood stream infected samples. These high multi resistance would be the production of hydrolytic enzymes and the acquisition of resistant mechanism by *Pseudomonas aeruginosa* strains. Our results were compared with the author Syed et al[11] mentioned in their study isolated the *Pseudomonas aeruginosa* from the different infection site and also proved the strain *Pseudomonas aeruginosa* showed multi drug resistant to currently prescribed antibiotics.

CONCLUSION

In this study concluded that the antibacterial activity of the synthesized silver nanoparticles was checked against blood stream infection causing multidrug resistant *Pseudomonas aeruginosa*. The antibacterial activity of silver nanoparticles such as $AgNo_3$ has maximum effect on blood stream pathogen. This biological approach toward the synthesis of silver nanoparticles using brown algae associated microbe has been exhibiting promising degrading activity to world killer pathogen and also the synthesis of nano drug were existence trailed over the periods, but their development was found to be luxurious and the use of numerous toxic chemicals for their synthesis makes the biological synthesis the more ideal option. So for this reason accompanying in this research focused to synthesis of nanoparticle from bio medically active marine seaweed from Thoppukadu Theevu Rameswaram for biosynthesis of nano drug. In spite bacterial can be used for nano silver synthesis because this type of formation is very easy, reliable and nontoxic in nature. In this regard data providing a potential platform for developing cancer theranostics of medicine

which combines specific targeted therapy based on specific targeted diagnostic tests are critically evaluated with a particular emphasis on this developed nano drug to overcome delivery barriers in upcoming.

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Conflicts of Interest – None.

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