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Research Article

Pharmacological activity of sea cucumber *Cucumaria frontosa* derived amylase enzyme against blood stream pathogen

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Abstract

At presently availability of resistance pathogen to numerous drugs is a common feature of hospital-acquired *Pseudomonas aeruginosa* strains. So, the present study collect the 80 blood sample from blood cancer patient in Revathi Medical Hospital, Tirupur City for isolating the PA strain from blood sample and confirmed the bacterial strain using standard test method followed by drug susceptibility test for identification of resistant's. In this study the maximum resistant was recorded in antibiotics Vancomycin 99% and maximum sensitive was observed in antibiotic Levofloxacin 99%. The blood stream infection causing PA plasmid extracted in 19 carcinogenic isolates which was showed more than 50% resistance selected for plasmid isolation. Due to the ability of resistant mechanism present in BSI pathogen the identification of new resources for novel antibiotics used urgently need to carry out a specific task to deal the blood stream infection. Moreover the enzyme especially the amylase have been isolated and purified from marine sea cucumber associated microorganism and the antimicrobial assay was done by using well techniques. The maximum zone of inhibition at 50, 100 and 150µl (, 25mm, 26mm and 16mm, 22mm, 23mm) of amylase extract was observed in the PAVG43,60 isolates. So hence this motto of this research proved the sea cucumber associated extracted amylase enzyme play a major role in the diagnosis of blood stream infection in cancer patients.

Keywords: Blood Stream Infection, *Pseudomonas aeruginosa*, Sea Cucumber, Amylase

1. INTRODUCTION

Today blood stream infection is major dreadful disease among human beings especially caused by gram negative bacterium *Pseudomonas aeruginosa.* Investigate from the Ghana and South Africans affects between 4 and 6.5% because of the blood stream infection (BSI) causing pathogen *Pseudomonas aeruginosa* [1].

Typically the BSI strain causes health associated infection among children as well as human beings with malignant disease associated with impaired protection mechanism [2]. The community spread disease may manifest in infants with other immune deficiency disorders it includes hypo gamma globulinaemia and neutropaenia disease [3]. The blood stream infection causing *Pseudomonas aeruginosa* also been reported among healthy young kids without any primary medical conditions [4]. In, some finds from Taiwan and Argentina country the fatality rate between 30 and 35% [5].

In recent years, Europe and United States indicated that 65% to 80% of gram negative infection (Entero bacteriaceae – family) observed in patients. Illness with BSI pathogen is very vital position as of utmost concern in critically and immune compromised patients who have been hospitalized for widespread period of time and have established broad-spectrum antimicrobial blood cancer therapy [6]. In this situation we need greater than 20% peak among the BSI patients receiving the starting step for antimicrobial treatment [7]. The appearance of antibiotic resistance drastically limits therapeutic options for *Pseudomonas aeruginosa* [8].

Today the marine enzymes have accelerated a metabolic process which is predominantly found in the sea cucumber to carry out a specific task to play lytic mechanism for diagnosis various treated diseases [9]. So far, the enzymes have gained attention by researchers throughout the world because they play not only *invivo* but possess potential industrial applications too. They have been screened extensively to isolate life saving drugs or biologically active substances all over the world. Since amylases enzymes have great demand of therapeutic pharmaceuticals, leather, laundry, food and waste processing industries, effectiveness of their production is very much targeted today. For this reason the goal of this research deals with a background intended to evaluate the enzyme activity from associated microbes of marine sea cucumber using different sources to eliminate the blood stream infection causing *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

2.1 Sample Collection

The carcinogenic blood was collected aseptically on citrated vacutainer from Revathi Hospital in Tirupur District and then the samples were inserted in to tube containing 5 ml of brain heart infusion broth using transport media for isolation of BSI causing carcinogenic pathogen.

2.2 Isolation and identification of BSI pathogen

The blood stream infection causing *P. aeruginosa* was isolated from the clinical blood samples. The PA was plated on Cetrimide agar medium. Further the plate was incubated at 37ºC. After day verifies the plates and record result if the change in color from Pale Green to Prussian Blue indicated the presence of *P. aeruginosa* in the collected samples. The BSI strain confirmed by 16SrRNA gene sequencing analysis.

2.3 Antibiotic Susceptibility Test

In this study BSI isolate susceptibility test done by the disc diffusion method of Kirby Bauer The nineteen antibiotic disks were used which include Kanamycin, Amikacin, Imipenam, Meropenam, Cephalexin, Erythromycin, Vancomycin, Carbenicillin, Tetracycline, Levofloxacin, Trimethoprim, Ofloxacin, Penicillin, Cefodoxime, Co-trimoxazole, Novobiocin, Amoxyclov, Norfloxacin and Piperacillin were applied on the seeded MHA plates in sterile condition. The susceptible and resistant inhibitory zone diameter breakpoint used throughout the study was ≥ 1.5 mm indicating sensitivity and ≤ 1.5 mm indicating resistance based on CLSI 2006 recommendation sensitivity pattern was compared with standard *P. aeruginosa* (NCTC, 10662).

2.4 Isolation of BSI plasmid

In this study the isolation of BSI plasmid done by the method of boiling preparation [10,11].

2.5 Isolation and identification of novel amylase enzyme from Sea Cucumber

Marine sea cucumbers were collected by hands picking in period of March – April 2021 from the Islands for the first time from Mandapam, Rameshwaram Coast. During the collection, marine sea cucumber *Cucumaria frontosa* were put into ziplock plastic bags and placed in a cool box and transported to research laboratory.

2.6 Laboratory Analysis

The collected marine sea cucumber (*Cucumaria frontosa)* were put into a sterile plate were initially, and the marine sea cucumber 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 gut of sea cucumber was dissected out and grind well using mortar and pestle for extraction of gut associated microbe. 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.

2.7 Isolation and identification of Bioactive Potential Bacteria Associated with Marine Sea Cucumber *Cucumaria Frontosa*

In this study, the biomedical active two bacteria namely *Bacillus megaterium* from marine sea cucumber were collected from Mandapam in the south east coast of India, Rameswaram, Tamil Nadu State, India by growing in marine agar media for specific isolation of pharmaceutically active bacteria and the identification done by phenotypic characterization of isolates.

2.8 Screening of Marine *Bacillus megaterium* for Amylase Production

The marine bacteria *Bacillus megaterium* were screened for amylase manufacture on Starch Agar medium at 37°C for 24 h. After incubation, starch agar was flooded with 1% iodine. Wait for 5 Minutes the amylase enzyme producing strain exhibiting clear zone around the bacterial colony.

2.9 Enzyme Production from Bacillus megaterium

Enzyme production was carried out by inoculating 10 ml of bacterial inoculums in 500 ml amylase production medium and it was kept on rotary shaker for 24 h of incubation [12]. At the end of the incubation extract the enzyme with the help of the centrifugation process finally the enzyme tested against BSI Pathogen.

3.0 Antibacterial activity of amylase enzyme against BSI pathogen

The antibacterial activity of amylase enzyme executed by diffusion assay against BSI pathogen *Pseudomonas aeruginosa*. The enzyme 50, 100 and 150µl concentration of amylase enzyme was treated against the BSI pathogen on MHA agar plate. Then the plates incubated 24 hours after end of the incubation the zone of inhibition measured by scale mm in diameter.

RESULT AND DISCUSSION

Totally 100 Blood Stream Infection samples were collected from blood cancer patients in Primary Health Centre around Tirupur Dt. 80 isolates of PA were isolated [Table 1, Fig 1]. The BS Infecting organism *P. aeruginosa* strains were confirmed by standard biochemical test of [Plate 1, Table 2] and genomic characteristics. In this research *P. aeruginosa* agar and Cetrimide agar media were used to isolate BSI pathogen.

Table 1: Prevalence of *Pseudomonas aeruginosa* based on gender

S. No	Gender	Prevalent sample
01.	Male	40
02.	Female	40
Total		80

Table 2: Biochemical characterization of *Pseudomonas aeruginosa*

S. No	Reaction	Result
01.	Indole	Negative
02.	Urease	Positive
03.	Glucose	Positive
04.	Lactose	Positive
05.	Sucrose	Positive
06.	Maltose	Positive
07.	Fructose	Positive
08.	Motility	Positive
09.	Catalase	Positive
10.	Oxidase	Positive
11.	Mannitol	Positive
12.	Raffinose	Positive
13.	Galactose	Positive
14.	Arabinose	Positive
15.	Methyl red	Positive
16.	Gram Staining	Negative
17.	Voges Proskauer	Positive
18.	Citrate utilization	Positive
19.	Nitrate reduction	Positive
20.	Starch hydrolysis	Positive

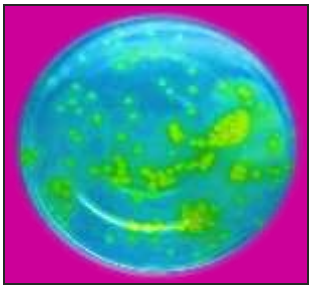

Plate 1: Isolated colonies of *Pseudomonas aeruginosa* from blood cancer patients

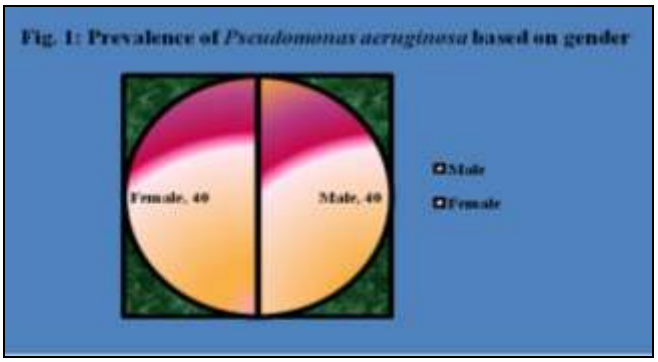

Figure 1: Prevalence of *Pseudomonas aeruginosa* based on gender

In this study there are different group of 20 antibiotics isolates were assayed against 80 isolates of *P. aeruginosa*. In this study 80 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%), Levofloxacin (1%), Trimethoprim (16.25%), Ofloxacin (50%), Penicillin (94%), Cefodoxime (73%), Oxacillin (80%), Co – Trimoxazole (94%), Novobiocin (80%), Amoxyclov (91.25%), Norfloxacin (95%) and Piperacillin (49%) [Table 3]. Result observed among them more than 18 antibiotics were showed highly resistant and only 2 antibiotics were showed sensitive out of 20 antibiotics [Table 5]. The maximum resistant was recorded in antibiotics such as Vancomycin 99% and maximum sensitive was observed in antibiotic such as Levofloxacin 99% [Fig 2]. The research agreed with the author [13] states that the imipenem resistant mechanism found in beta lactam producing *Pseudomonas aeruginosa* totally attributed to expression of outer layer membrane protein Resistance to imipenem has been found to be independent of β-lactamase production in *P. aeruginosa* it has been attributed to diminished expression of some outer membrane proteins. Totally in their study more than 55% of BSI isolates were sensitive to imipenem antibiotic got 56% sensitive beside the imipenem antibiotic.

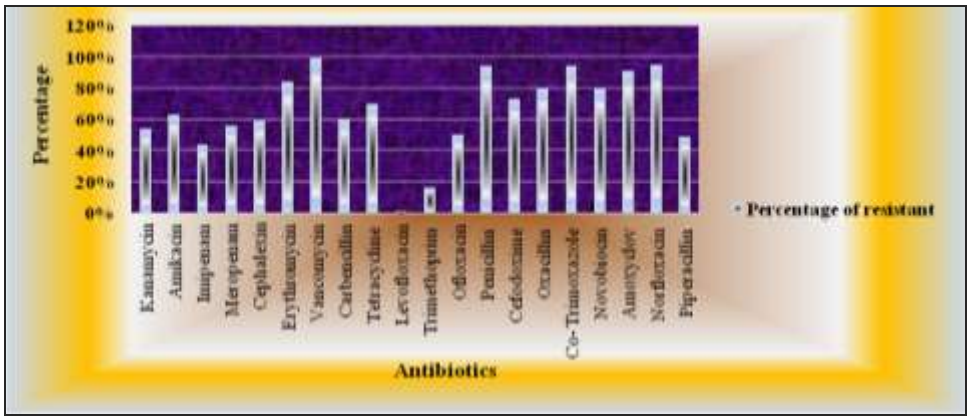

Figure 2: Antibiotic resistant percentage of *Pseudomonas aeruginosa*

The antibiogram pattern of all isolates carcinogenic *P. aeruginosa* strains were performed against 20 frequently prescribed antibiotics currently used to treat blood cancer infections (Leukemia). A total of 80 strains the maximum percentage 90% was recorded in strain no PAVG43 and the minimum resistant pattern percentage (50%) was recorded in strain no PAVG67.

In order to understand that all carcinogenic *P. aeruginosa* isolates showed multiple antibiotic resistances. Such that one isolate showed resistant against 10, 17 and 18 types of antibiotics, 2 isolates showed resistant against 16 antibiotics, nine strains resistant 15 types of antibiotics, fourteen strains resistant against 11 types of antibiotics and finally sixteen strains resist against 12 types of antibiotics, 13 and 14 types of antibiotics resist against seventeen carcinogenic organism. In this study maximum MAR index 0.90 was showed by PAVG43 and minimum MAR index 0.50 was showed by PAVG67 [Table 6]. All the 80 isolates of MRPA were showed MAR index in between these ranges. This result is agreed with the scientist [14] stating that the MAR index higher than 0.2 indicates the isolation of organism from environment where antibiotics were often used.

In this research, nineteen carcinogenic isolates (PAVG01, PAVG02, PAVG03, PAVG05, PAVG06, PAVG21, PAVG41, PAVG43, PAVG44, PAVG47, PAVG51, PAVG56, PAVG58, PAVG60, PAVG61, PAVG63, PAVG64 and PAVG80) which was showed more than 50% resistance against tested antibiotics were selected for plasmid isolation.

Two fragments were obtained in Strain No. PAVG80 with molecular weight of 10,000bp and 6,000bp respectively, followed by the Strain No. PAVG63 and PAVG64 were harbored two fragments of molecular weight 10,000bp and 4,000bp followed by the Strain No. PAVG60, PAVG61 and PAVG62 were harbored two fragments of molecular weight 10,000bp and 3000bp followed by the Strain No. PAVG05, PAVG44, PAVG47, PAVG51, PAVG56 and PAVG58 were harbored two fragments of molecular weight 10,000bp and 2,000bp followed by the strain No. PAVG06 were harbored two fragments of molecular weight 10,000bp and 1,000bp followed by the strain No. PAVG01 and PAVG02 were harbored two fragments of molecular weight 10,000bp and

500bp followed by other three isolates PAVG03, PAVG21 and PAVG41 were harbored one fragment of molecular weight 1500bp respectively. Molecular weight of the fragments was estimated by using 10,000bp DNA ladder (Medox, Chennai), Multidrug resistance bacteria were found to be carrying plasmids [Plate 2, 3].

Table 3: Antibiotic resistant percentage of *Pseudomonas aeruginosa*

S. No	Antibiotics	Percentage of resistant
01.	Kanamycin	54%
02.	Amikacin	63%
03.	Imipenam	44%
04.	Meropenam	56%
05.	Cephalexin	59%
06.	Erythromycin	84%
07.	Vancomycin	99%
08.	Carbenicillin	60%
09.	Tetracycline	70%
10.	Levofloxacin	1%
11.	Trimethoprim	16.25%
12.	Ofloxacin	50%
13.	Penicillin	94%
14.	Cefodoxime	73%
15.	Oxacillin	80%
16.	Co- Trimoxazole	94%
17.	Novobiocin	80%
18.	Amoxyclov	91.25%
19.	Norfloxacin	95%
20.	Piperacillin	49%

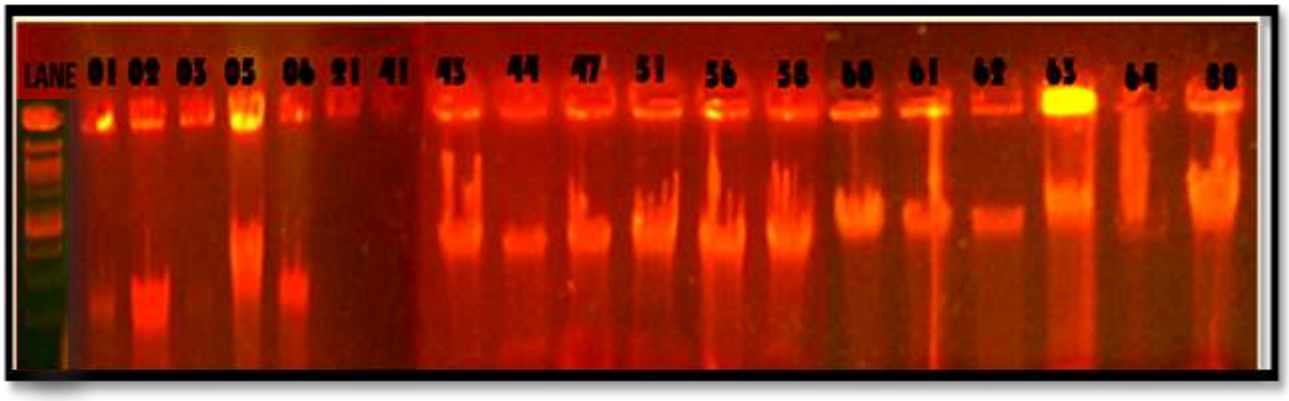

Plate 2: Isolation of plasmid from *Pseudomonas aeruginosa*

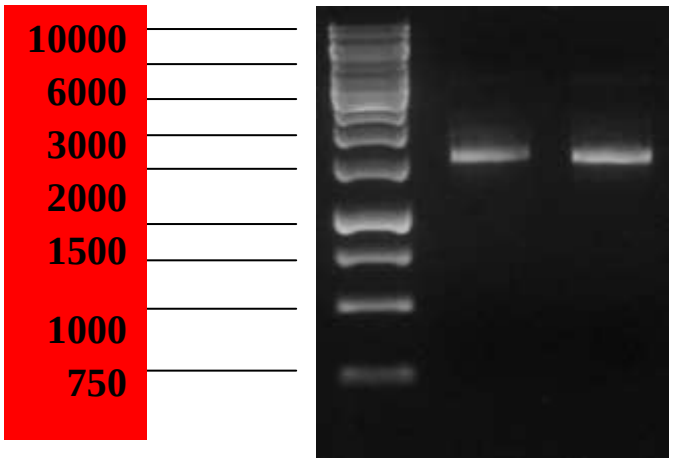

Plate 3: PCR amplification of biofilm producing *Pseudomonas aeruginosa*

Recently, there has been increasing interest in the screening of bioactive compounds from nature has been playing an important role in providing therapeutic entities since ancient time for treating and preventing human disease. The vast sources of nature includes terrestrial and marine plant, microorganisms, vertebrates and invertebrates etc. Additionally, amylase enzymes have been used for a long time in various forms of therapy. Their use in medicine is notable based on several clinical studies indicating that main defense against blood stream infection causing pathogen.

In this study, marine bacteria namely *Bacillus megaterium* was isolated from marine sea cucumber - *Cucumaria Frontosa* [Plate 4] are collected from Mandapam coast of India and the *Cucumaria Frontosa* identified at CMFRI for the production of amylase enzyme for the treatment of BSI.

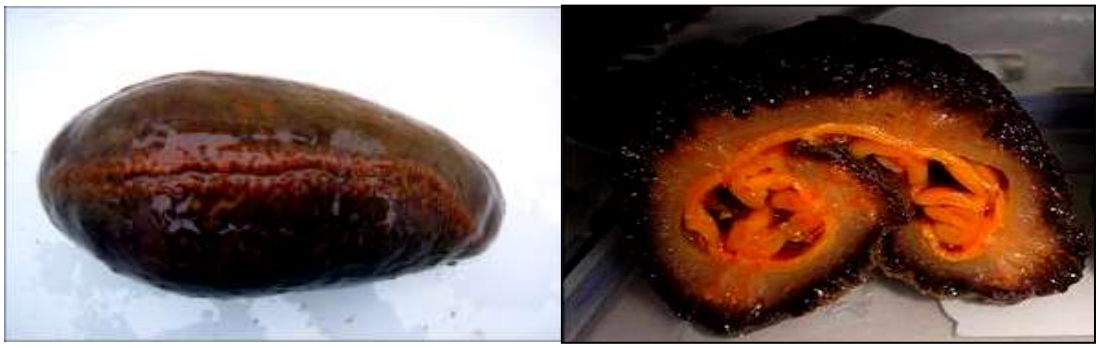

Plate: 4 Marine Sea Cucumber *Cucumaria Frontosa*

A total of 20 isolates from each different sample were confirmed as epiphytic bacteria like *Bacillus megaterium* from marine sea cucumber *Cucumaria frontosa*. All identification the selected colonies were streaked on starch agar and gelatinase agar plates for the production of pharmaceutically active enzymes. It was incubated at 37° C, 24hrs. The maximum zone of inhibition producing isolates subjected to extracellular enzymes production.

The scientist [15] was conducted to find out bioactive compounds in sea cucumbers collected from Karimunjawa as anti-microbial agents beside several pathogenic microorganisms. In their study the research using five different sea cucumbers species were Stichopus variegatus, Stichopus chloronotus, Bohadschia mamorata, Stichopus herrmanni and Bohadschia argus. Novelist using sea cucumbers extract were subjected to anti-microbial tests using *Staphylococcus aureus, Escherichia coli, Vibrio anguila, Vibrio voinivica, Bacillus subtilis, and Pseudomonas sp*. Their finding agreed with this present study using 3 different concentrations (50µg, 100µg and 150µg) of sea cucumber extract derived amylase enzyme were used in this assay against leukemia causing *Pseudomonas aeruginosa* using well assay technology. The maximum zone of inhibition 11mm, 25mm, 26mm and 16mm, 22mm, 23mm were observed in amylase producing strain no. PAVG43 and PAVG60 [Plate 5]. The study proved the sea cucumber derived amylase enzyme able to cleave the pathogenic bacteria cell wall. It act as a good therapeutic drug to treat the blood stream infection causing blood cancer isolate *Pseudomonas aeruginosa*.

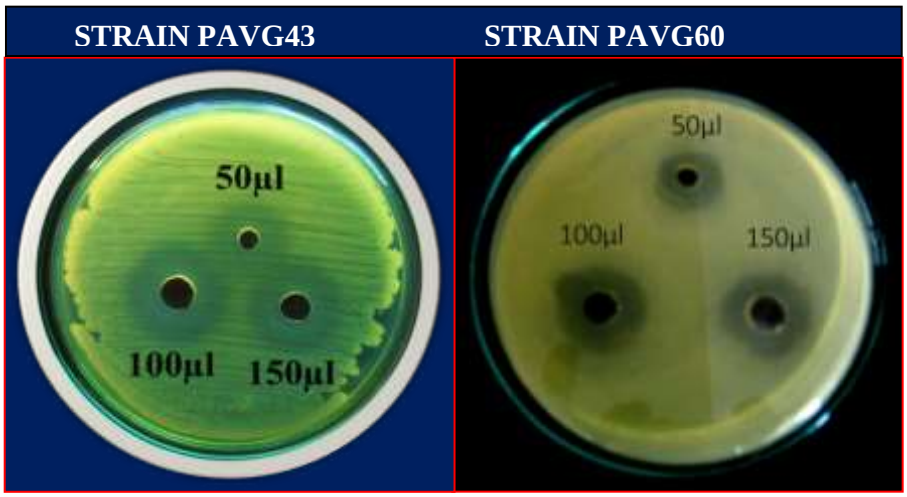

Plate: 4 Antibacterial activity of amylase enzyme against BSI pathogen

CONCLUSION

The present research concluded that the marine *Sea Cucumber – Cucumaria Frontosa* is ability to synthesis amylase enzyme from gut extract of sea cucumber is radically valuable drug beside BSI pathogen. Hence these therapeutic amylase enzyme extracts act as broad microbial activity beside to kill the BSI pathogen around blood cancer community people and this findings give surety that there is no any side effect at the time of treatment of blood stream infection. So this current research paper provides evidence to display a choice of therapeutic approach it makes it important biomedical

applications for treating all blood stream infection future around blood cancer community.

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AUTHORS CONTRIBUTIONS

Each author has given considerable and equal contributions to this research

CONFLICTS OF INTEREST

The authors have no conflict of interest

AUTHORS FUNDING

Not applicable

REFERENCES

1. Lochan H, Pillay V, Bamford C, Nuttall J and Eley B. Bloodstream infections at a tertiary level paediatric hospital in South Africa. BMC Infect Dis. 2017; 17(1):750. https://doi.org/10.1186/s12879-017-2862-2

2. Viola L, Langer A, PulitanÒ S, Chiaretti A, Piastra M and Polidori G. Serious Pseudomonas aeruginosa infection in healthy children: case report and review of the literature. Pediatr Int. 2006; 48(3):330-333. https://doi.org/10.1111/j.1442-200X.2006.02214.x

3. Sanford E, Farnaes L, Batalov S, Bainbridge M, Laubach S, Worthen HM, Tokita M, Kingsmore SF and Bradley J. Concomitant diagnosis of immune deficiency and Pseudomonas sepsis in a 19 month old with ecthyma gangrenosum by host whole-genome sequencing. Mol Case Stud. 2018; 4(6):32-44 https://doi.org/10.1101/mcs.a003244

4. Kuo KC, Kuo HC, Huang LT, Lin CS and Yang SN. The clinical implications of ABO blood groups in Pseudomonas aeruginosa sepsis in children. J Microbiol Immunol Infect. 2013; 46(2):109-114. https://doi.org/10.1016/j.jmii.2012.01.003

5. Rosanova MT, Mussini MS, Arias AP, Sormani MI and Sarkis C (2019). Epidemiological features and risk factors for mortality in Pseudomonas aeruginosa bacteremia in children. Arch Argent Pediatr. 2019; 117(2):128-131. https://doi.org/10.5546/aap.2019.eng.128

6. Pollack M. Pseudomonas aeruginosa. In: Principles and practice of infectious diseases (eds. G.L. Mandell, J. E. Bennett and R. Dolin), Churchill Livingstone, Philadelphia, PA, USA, 5th Ed, 2008. P. 2310 - 2335.

7. Wisplinghoff H, Seifert H, Tallent SM, Bischoff T, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features, and susceptibilities. Pediatr. Infect. Dis. J. 2003; 22:686 - 691. https://doi.org/10.1097/01.inf.0000078159.53132.40

8. Shamima Nasrin, Nicolas Hegerle, Shaichi Sen, Joseph Nkeze, Sunil Sen, Jasnehta Permala Booth, Myeongjin Choi, James Sinclair, Milagritos D, Tapia J, Kristie Johnson, Samba O, Sow, Joshua T, Thaden, Vance G, Fowler Jr, Karen A, Krogfelt, Annelie Brauner, Efthymia Protonotariou, Eirini Christaki, Yuichiro Shindo, Andrea L, Sadia Shakoor, Ashika Singh Moodley, Olga Perovic, Jan Jacobs, Octavie Lunguya, Raphael Simon, Alan S, Cross and Sharon M, Tennant. Distribution of serotypes and antibiotic resistance of invasive Pseudomonas aeruginosa in a multi-country collection. BMC Microbiology. 2022; 22(13):1 - 12. https://doi.org/10.1186/s12866-021-02427-4

9. Neelam Gurung, Sumanta Ray, Sutapa Bose and Vivek Rai. A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. Biomed Research International. 2013; 1 - 18. https://doi.org/10.1155/2013/329121

10. David Holmes, S, Michael Quigley, A rapid boiling method for the preparation of bacterial plasmids. Analytical Biochemistry. 1981; 114(1):193 - 197. https://doi.org/10.1016/0003-2697(81)90473-5

11. Riggs MG, McLachlan A. Biotechnology Techniques. 1986; 4:310 - 313. https://doi.org/10.1016/0734-9750(86)90419-2

12. Ashwini K, Gaurav Kumar, Karthik L, and Bhaskara Rao KV. Optimization, production and partial purification of extracellular α amylase from Bacillus sp. marini. Achieves of Applied Science and Research. 2011; 3(1):33 - 42.

13. Buscher KH, Cullman W, Dick W, Wendt S, Opferkuch W. Imipenem resistance in P. aeruginosa is due to diminished expression of the outer membrane proteins. J. Infect. Dis. 2000; 156:681- 685. https://doi.org/10.1093/infdis/156.4.681

14. Paul S, Bezbarauh RL, Roy MK and Ghosh AC. Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. Lett. Appl. Microbiol. 1997; 24:169-171. https://doi.org/10.1046/j.1472-765X.1997.00364.x

15. Delianis Pringgenies. Antibacterial Activity of Sea Cucumbers Harvested from Karimunjawa Aktivitas Antibakteri Teripang yang dipanen dari Karimunjawa. Squalen Bulletin of Marine & Fisheries Postharvest & Biotechnology. 2013; 8(2):87-94 https://doi.org/10.15578/squalen.v8i2.90