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Effect of *Pseudomonas Aeruginosa* Extract on Human Blood Cells

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Abstract

This study was conducted to observe the effect of *Pseudomonas aeruginosa* extract on different human blood cells with different interval of time. Venous blood was collected from laboratory personnel in EDTA tube. *Pseudomonas aeruginosa* was isolated from different clinical samples. 20 different isolates were cultured on fresh nutrient agar after 24 hours of incubation at 37^{0}C. Isolates were centrifuged. The bacteria were resuspended in cold Sterile Distilled Water (SDW) and disrupted by sonication on ice for three times after 1 min intervals. Extract filtered through 0.22 µm Millipore Millex. Extracts were incubated with human blood. After incubation time, blood was used to observe effect on extract, on cells in cell counter. This study shows the effect of *Pseudomonas aeruginosa* extract on human blood cells. In this study, 20 Non-duplicate isolates of Non-MDR, MDR and PDR Pseudomonas aeruginosa were tested. Blood Sample (Heparinized venous blood) was collected in EDTA tube from healthy personnel. Bacterial isolation and further processing of extraction of toxin was done as per the standard procedure. Significant difference of *Pseudomonas aeruginosa* extract of all three groups was seen on human blood cells. Effect of extract was same on blood cells at particular interval of time.

Keywords: *Pseudomonas Aeruginosa*; Extract; Venous Blood; Blood Cells; Toxin Effect.

Introduction

P. aeruginosa is a non-fermentative Gram negative bacteria widely distributed in nature and can survive on a wide variety of surfaces and in hospital environment [1].

Pseudomonas aeruginosa is known to cause a wide spectrum of diseases. It can infect almost any external site or any internal organ, and therefore can be isolated from various clinical samples such as pus, sputum, urine, blood etc [2,3].

Pseudomonas aeruginosa is a leading cause of nosocomial as well as wound infections such as burn wounds. It is responsible for 10% of all hospital-acquired infections. Infections caused by *P. aeruginosa* are often severe and life threatening and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of an emergence of antibiotic resistance during therapy thus resulting in severe adverse outcomes [4,5].

Pseudomonas aeruginosa acquired resistance to readily conventional antimicrobials following intensive use of antimicrobials [6]. It can be categorized as Multi-drug resistant (MDR) and Pan-drug resistant (PDR).

Multi-drug resistance is defined as non-susceptibility to at least one agent in three or more antimicrobial categories [7].

Pan-drug resistance is defined as isolates intermediately-resistant or totally resistant to all antimicrobial agents available for clinical use according to routine disk diffusion susceptibility results [8,9]. The prefix "pan" has its origin in the ancient Greek language, meaning "all" or "whole" [10].

Toxigenesis or the ability to produce toxins is an underlying mechanism by which many bacterial pathogens produce disease [11]. Toxins are virulence determinants that play an important role in microbial pathogenicity and evasion of the host immune response. This makes them ideal targets for the development of novel antimicrobial strategies. The potential applications of toxin research extend beyond simply combating microbial pathogens and include use as novel anti-cancer drugs and other front-line medicines and as tools in neurobiology [12].

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Materials and Methods

Bacterial Extract Preparation [13]

Collection of Blood Sample

Heparinized (20 ml) venous blood was collected in EDTA tube from healthy personnel.

Procedure

The bacterial population isolated from aqueous extracts. The bacteria were removed from the culture medium by centrifugation at 10,000 rpm for 1 hour. The bacteria were further processed, using one of the following a procedure:

In this procedure, the bacteria were resuspended in cold Sterile Distilled Water (SDW) and disrupted by sonication on ice for three times after 1 min intervals. This was followed by centrifugation at 10,000 rpm for 1 hour and lyophilisation of the pellet and supernatant and finally filtered through 0.22 µm Millipore Millex.

Effect of Extract on Blood Cells

In this study, 10 Non-MDR, 08 MDR and 02 PAN-drug resistances of *Pseudomonas aeruginosa* (PSA) isolates were tested.

Procedure

1. Run 0.5ml blood samples (normal blood) in coulter counter from EDTA tube, considered as positive control.
2. Add 0.2 ml extract in 2 ml blood for MDR and Non-MDR PSA isolates and incubate at 37^{0}C for 1 hour.
3. Take first reading of the entire sample in coulter cell counter after 1hour incubation.
4. Incubate the entire sample for 2 hours at 37^{0}C.
5. Take second reading of entire sample.
6. Incubate the entire sample for 3 hours at 37^{0}C.
7. Take third reading of entire sample.

Results

Table 1: Descriptive statistical analysis of in vitro effect of Non-MDR *Pseudomonas aeruginosa* extract on human blood cells

Parameter	Control	At 1 Hour	At 2 Hour	At 3 Hour	P value
WBC Count	6.8	5.57(0.25)	4.5(0.28)	3.51(0.28)	< 0.001
Hb Content	14.8	12.2(0.29)	10.54(0.26)	9.24(0.18)	< 0.001
Platelet Count	15.4	13.15(0.09)	11.66(0.36)	10.18(0.2)	< 0.001

** Significant at 1 % level, Units: WBCs: 10^3/mm^3, Hb: gms/dl, PLTs: 10^4/ mm^3, MDR: Multi Drug Resistance

Table 2: Descriptive statistical analysis of in vitro effect of MDR *Pseudomonas aeruginosa* extract on human blood cells

Parameter	Control	At 1 Hour	At 2 Hour	At 3 Hour	P value
WBC Count	6.8	5.2(0.6)	4.2(0.47)	3.1(0.59)	< 0.001
Hb Content	14.8	12.31(0.28)	9.97(0.58)	8.65(0.48)	< 0.001
Platelet Count	15.4	12.45(0.6)	11.16(0.7)	9.47(0.46)	< 0.001

** Significant at 1 % level, Units: WBCs: 10^3/mm^3, Hb: gms/dl, Platelets: 10^4/ mm^3, MDR: Multi Drug Resistance

Table 3: In-vitro effect of PDR *Pseudomonas aeruginosa* extract on human blood cells

Parameter	Control	At 1 Hour	At 2 Hour	At 3 Hour	P value
WBC Count	6.8	5.65(0.15)	4(0.1)	2.9(0.1)	< 0.001
Hb Content	14.8	11.5(0.7)	9.9(0.8)	8.7(0.4)	< 0.001
Platelet Count	15.4	12.15(1.0)	10.95(0.85)	9.6(0.3)	< 0.001

** Significant at 1 % level, Units: WBCs: 10^3/mm^3, Hb: gms/dl, Platelets: 10^4/ mm^3, PDR: Pan-Drug Resistance

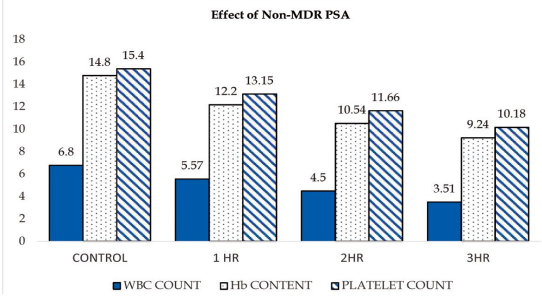

Fig. 1: In-vitro effect of Non-MDR *Pseudomonas aeruginosa* extract on human blood cells

Units: WBCs: 10^3/mm^3, Hb: gms/dl, Platelets: 10^4/ mm^3, MDR: Multidrug Resistant *Pseudomonas Aeruginosa.*

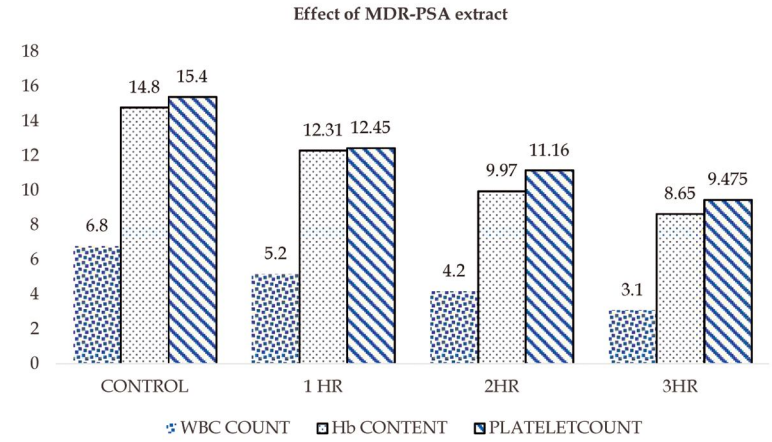

Fig. 2: In-vitro effect of MDR PSA extract on human blood cells

Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, MDR, MDR: Multidrug Resistant

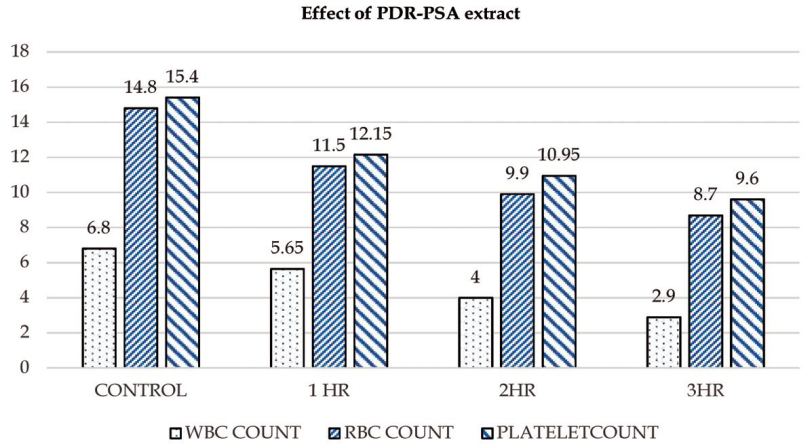

Fig. 3: In-vitro effect of PDR PSA extract on human blood cells

Units: WBCs: $10^3/mm^3$, Hb: gms/dl, Platelets: $10^4/ mm^3$, MDR, MDR: Multidrug Resistant

Discussion

Effect of Pseudomonas Aeruginosa Extract on Blood Cells

Aldona Baltec et. al. (1985), New York, studied effect of *Pseudomonas aeruginosa* cytotoxin on human serum and granulocyte. It was found that the lytic effect of *Pseudomonas aeruginosa* on Neutrophils was directly related to the concentration of cytotoxin and time of exposure [14].

Pseudomonas aeruginosa cytotoxin was found to agglutinate human erythrocytes in addition to its erythrocyte-agglutinating activity [15]. Therefore in present study we studied the effect of cytotoxin on hemoglobin content instead of effect cytotoxin of RBC count.

In the present study, effect of *Pseudomonas aeruginosa* extract showed rapid effect on blood cells in both MDR PSA and Non-MDR PSA.

Cell counts and hemoglobin content were decreasing steadily for interval of time in all three groups; MDR PSA, Non-MDR PSA and PDR which is statistically significant. This proves the cytolytic effect of *Pseudomonas aeruginosa* extract of blood cells.

Burn patients having bluish green discharge from their wounds is the most common and most serious life threatening infection; such patients require treatment with combination of higher antibiotics. In spite of such a massive treatment patients may not survive because of hemolytic effect of pseudomonas toxin.

Conclusion

Effect of extract of Non-MDRPA, MDRPA and PDRPA on human blood cells was studied.

Extract caused significant decrease in blood cells with respect to time interval.

Cytolytic effect of *Pseudomonas aeruginosa* extract contains cytotoxin produced by organism plays important role in pathogenesis of sepsis and opportunistic infections.

In spite of aseptic precautions; infection with Pseudomonas *aeruginosa* is frequently seen as this microorganism is highly resistant to antibiotics also mechanisms of pathogenesis are not clearly understood. Therefore this study suggest that along with combination of higher antibiotics whole blood transfusion should be given which will help the patient to withstand patient's immunity and mortality rate of patients infected with pseudomonas may decrease. This study also suggests need of further detail research to overcome the island of *Pseudomonas aeruginosa* resistance. Immunotherapy in human burns cases with antiserum to *Pseudomonas aeruginosa* may be useful. Further research study should be done to find out pathogenesis of infection caused by *Pseudomonas aeruginosa* also to find out *Pseudomonas aeruginosa* vaccines to combat this life threatening microorganism.

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