P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Short Communication

Hematological Variations of Mice Inoculated by Serratia marcescens Whole Cell Sonicated Antigens

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Article History: Received: April 07, 2018 Revised: July 29, 2018 Accepted: August 13, 2018

ABSTRACT

The current study was planned to make antigens of sonicated *Serratia marcescens* and study its result on the mice blood cells for the first time in Iraq. For this purpose, 24 mice divided into four groups. The first group was vaccinated with killed whole cell sonicated antigens of *Serratia marcescens* (KWCSA-SM) (1000 µg/ml) Subcutaneously. Second group was immunized with killed (KWCSA-SM) (500 µg/ml) S/C. Third group was immunized with whole cell *Serratia marcescens* (1.5×10^8cfu/ml), 5th group was injected by P.B.S. (pH7.2) as negative control group. Blood cells showed variances in the hematological changes between the 1st group and the other immunized groups associated with negative control group that show no hematological variations in their cells. Statistical study exposed significant differences between groups ($P< 0.05$). The aim of this study is to observe the effect of the two antigens concentration on the hematological changes of blood cells for the first time in Iraq.

Key words: Serratia marcescens, Antigens, Hematological Changes, Mice

INTRODUCTION

Serratia species are opportunistic gram-negative bacteria considered as opportunistic pathogen. *Serratia* are common in the environment but are not a widespread component fecal flora of the human. (Donnenberg *et al.*, 2010)

Some strains of *S. marcescens* are capable of producing a pigment called prodigiosin. The chemical structure of prodigiosin has been unveiled, it was first used as marker in order to trace bacterial activity and transmission, antibodies and T-cells can be triggered by this pigment. (Mahlen, 2011)

Serratia are accomplished of successful in diverse environments, including water, soil, and the digestive tracts of various animals. (Carrero *et al*., 1995) *S. marcescens* has a preference for growing on starchy foodstuffs, where the pigmented colonies are easily false for drops of blood.

Serratia infection is responsible for about 2% of nosocomial infections of the bloodstream, urinary tract, respiratory tract, surgical wound. An occurrence of *S. marcescens* bloodstream contaminations was recognized in patients getting polluted bags of parenteral food. Grohskopf *et al.*, 2001) Occurrences of *S. marcescens* wound infections, arthritis and meningitis have happened in pediatric zones.

Also, as revealed in the cell structure, the Lipo polysaccharide layer is attached to the outer membrane of the Gram negative bateria. The Lipo polysaccharide performances as an endotoxin meaning a cell constituent that is inoffensive as long as the pathogen leftovers intact. The statement of Lipo polysaccharide would over-stimulate the host defenses and reason them to feel lethal endotoxic tremor (Ursua *et al*.,1996). The attendance of Lipo polysaccharide therefore creates it hard to kill *S. marcescens* without producing the death of the host's cells.

S.marcescens comprise these R-factors which are a specific kinds of plasmid transmits one or more genes that that discusses confrontation to different kinds of antimicrobial agents. The influence of R-factors to the confrontation of *Serratia* to numerous medications has been studied as far back as 1969 (Julie *et al*., 2009). Other trials have decided that the transmission system of R-factors in *S. marcescens* may be temperature sensitive and more probable to happen between those bacteria that are found to be more closely connected phylogenetically.

The G.I epithelium organizes numerous innate protection devices to fight bacterial, involving epithelial integrity, fast epithelial cell income, rapid exclusion of diseased cells, autophagy, and innate immune responses. And releasing specific cytokines such as IL-8 and TNF-alpha in reply to infection with invasive strain of by *S. marcescens* (Ursua *et al*.,1996).

Cite This Article as: Mohammedv, BN Nadhom and ST Mousa, 2018. Hematological variations of mice inoculated by serratia marcescens whole cell sonicated antigens. Inter J Vet Sci, 7(3): 159-161. www.ijvets.com

The outbreak of *S. marcescens* blood stream infection apparently through the usage of fluids and catheters contaminated with this bacteria (Mahlen, 2011).

Inoculation of *S. marcescens* into the blood (hemolymph) of the Bombyx mori, silkworm induced caspase initiation and apoptosis of hemocytes. This procedure reduced the innate immune response in which pathogen cell wall contents, such as stimulate hemocytes, glucan principal to the initiation of insect cytokine paralytic peptide. (Braun *et al*., 1991, Braun *et al*., 1992).

These answers propose that *S. marcescens* persuades apoptosis of host I.Cs via LPS- and flagella-dependent motility, leading to the destruction of host innate immunity.(Braun *et al.*, 1993)

The *S. marcescens* piliated strain motivated superoxide production of Polymorphic nuclear cells twice as much as the *S. marcescens* or non piliated strains did. The MS-piliated strain was more vulnerable to phagocytosis than was the MR- or non piliated strain. Those directed strains was more sensitive to phagocytosis which principal to tissue injury in infected organs (Ruan *et al*., 1990).

Almost all straining of *S. marcescens* conceal a cytotoxin (Braun *et al*., 1991, Braun *et al.*, 1992, Braun *et al*., 1993, Ruan *et al*., 1990) that reasons hemolysis of animal and human RBCs (Schiebel *et al*., 1989) and the secretion of the inflammatory mediators histamine and leukotrienes from leukocytes (König *et al*., 1987).

MATERIALS AND METHODS

Laboratory animals

Total number 24 mice of both genders which obtained commercially, were adapted for 1 week before started experiment by reared in separated clean and disinfected cages; they were fed on commercial assorted pellets and clean tap water.

S. marcescens

S. marcescens which was obtained from Microbiology Unit -Baghdad University - College of Veterinary Medicine strain named (ABH1) and the confirmatory diagnosis in (CPHL) Central Puplic Health Labrotory was established again according to API-20E system. API-20E system (Analytical profile index for Enterobacteriaceae test): (Atlas, 1995). The protein concentration of *S. marcescens* was measured by using Biuret method according to (AL-Tabaqchally, 2015).

Laboratory animal (mice) immunization

Twenty four mice of male and female were used which were randomly divided into five equal groups (6 animals for each group), as follows:

1. The 1st group was inoculated with one ml (1000µg/ml) of Killed Whole Cell Sonicated Antigen –*S.M* S/C.
2. The 2nd group was inoculated with one ml (500µg/ml) of Killed Whole Cell Sonicated Antigen – *S.M* S/C.
3. The 3rd group (positive control group) was injected with one ml of (1000µg/ml) of Killed Whole Cell Sonicated Antigen –*S.M* S/C.
4. The 4th group (negative control group) was inoculated with one ml PBS (PH 7.2) S/C.
5. At day 10 of immunization blood tasters were composed from the direct puncture of the heart by sterile syringes for blood picture.
6. At day 20, 40, 60, blood samples (3ml) were composed from all mouse groups for blood picture.
7. At day 60, challenge test was done by *S. marcescens* at dose (1.5×10^8cfu/ml) for each animal orally.

Blood samples

Blood samples (1 ml) were composed from the heart puncture of all animals at day 10, 20, 30, 40, 50, post immunization. Blood collected then kept in a slant position for few minutes until the clot formation and then separated by centrifuge at (3500 rapid per 10 minutes and the serum stored in (-20 ºC) according to (- Douglas *et al*., 2010).

Statistical analysis

Analysis of results was done by using two ways of classification with interaction method and program of SAS (Snedecor *et al*., 1980).

RESULTS AND DISCUSSION

Bacterial isolation results of one hundred and fifty milk tasters presented that 6 samples were give positive result to *S. marcescens* represented 4% from milk samples, (Di Guardo *et al*., 1997) showed that 4(3%) out of 120 cow affected by *S. marcescens* mastitis and these outcomes in contract with the present study.

Bacterial culturing revealed different morphological features of bacteria on diverse media, after incubation at 37°C for 24hours. On MacConkey agar colonies are lactose fermentor and appear red duo to the capability of *S. marcescens* to give red pigment as showed in Fig. (1) These marks as same with (Jawetz *et al*., 2007) isolated bacteria were showed under microscopic gram negative rods.

The Biochemical ID of *S. marcescens* showed that bacteria were Gram –ve, Rod, Catalase positive, oxidase negative, lactose non- fermenter, motile, Indole negative, citrate utilization positive, TSI y/y, DNase positive and Urease negative as (Quinn *et al*., 2004). To approve the diagnosis, RapID^{TM}ONE System and Api 20 E system.

There are hematological variations in the inoculated groups that equated with negative control that show no changes in their blood cells. In the group Our research designated to record the hematological changes produced by *S. marcescens* which caused cytotoxicity and morphologic variations in host cells.This pathogen showed expressively more epithelial cell attack compared with other gram negative bacteria such as E.Coli. as (Di Guardo *et al*., 1997).

Results of blood picture showed differences between groups, Statistical study was done using software (Statistical Analysis System - version 9.1), Two-way ANOVA and least significant differences (LSD) was done to assess significant differences among means. ($P<0.05$) was considered statistically significant as (Di Guardo *et al*., 1997) Table 1.

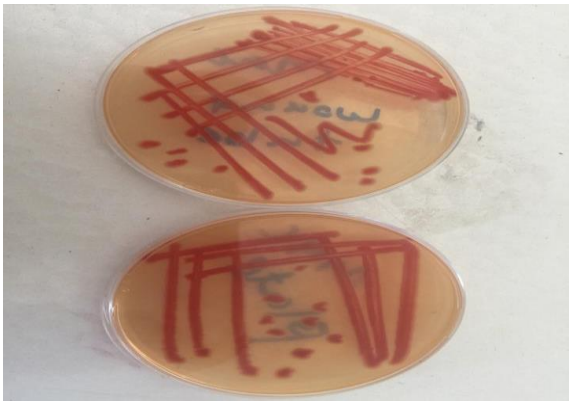

Fig 1: *S. marcescens* on nutrient agar produce red pigment.

Table 1: statistical analysis showed the effect of *S. marcescens* on blood cells of mice.

Group	1	2	3	4
A	B 0.90±0.09a	B 1.31±0.21a	B 1.15±0.10a	A 6.43±2.39a
B	A 1.36±0.21a	A 0.88±0.12a	A 1.42±0.07a	A 2.23±0.54c
C	A 0.99±0.08a	A 1.14±0.20a	A 0.83±0.07a	A 2.58±0.37bc
D	B 1.41±0.28a	B 1.70±0.15a	B 1.15±0.13a	A 4.35±1.61b
E	B 0.70±0.10a	B 0.93±0.18a	B 0.76±0.09a	A 3.17±0.60bc
LSD	1.9359			

Means with different small letter in the same column significantly different (P<0.05); Means with different capital letter in the same row significantly different (P<0.05); Different capital letters mean significant differences between isolates (P<0.05). [17] Institute that s. marcense has cytotoxic result on animal and human red blood cells as the same with the current study.

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