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Comparative study of serum protein activity of blood against *Shigella flexneri* in urban and slum population of Bangladesh

*** Fahareen Binta Mosharraf, Sarjana Bilkis Rahman, Salman Khan Promon, Iftekhar Mohammad Shafiqul Kalam, Mehnaz Karim**

Mathematics and Natural Science Department, BRAC University, Mohakhali, Dhaka, Bangladesh

Abstract
Complement are group of serum proteins which can be activated by antigen-antibody complexes or other substances, which may result in lysis of a microbial target, or a variety of other biological effects important in both innate and adaptive immunity. The last several years have seen an enormous expansion of parallel research on bacteria and the complement system and acquaint the role of complement proteins in biological phenomena. This study sought to find out the in vitro examination of bactericidal action complement proteins derived from blood sera of a defined group of urban and slum people against *Shigella flexneri* generating a critical problem in modern medical therapy for bacterial infections. This comparative study showed the susceptibility of clinical isolates of *Shigella flexneri* to bactericidal action of complement proteins of both urban and slum people blood serum. However the slum people indicated more effective complement mediated killing in comparison to urban people.

Keywords: serum protein, bactericidal activity, *shigella flexneri*

1. Introduction

1.1 About *Shigella* spp.

Shigella are gram-negative, non-motile, facultative anaerobic, non-sporulating, rod shaped bacteria that cause the disease shigellosis, which is also known as bacillary dysentery. Organisms of the genus *Shigella* belong to the *Enterobacteriaceae* family [1, 2, 3]. This group of bacteria was first described by and named after Japanese scientist Kiyoshi Shiga in 1898, after he isolated what he called *Bacillus dysenteriae* (now known to be *Shigella dysenteriae* serotype 1) from a patients' stool during a dysentery epidemic in Japan in 1897 [4, 5, 6]. *Shigella* infection is a major public health problem in developing countries with poor sanitation. Humans are the natural reservoir for this organism. Endogenous *Shigella* species are not present in any natural food products, but a wide variety of foods may be contaminated [7]. Transmission of the bacteria occurs by the fecal–oral route. *Shigella* species have a very low infective dose, as low as 10 to 100 organisms.

The bacterium *Shigella flexneri* is a causative agent of shigellosis, which is a severe infection of the colonic epithelium. It is primarily transmitted between hosts via the fecal-oral route to its infective site in the colon [8]. The organism enters the colonic epithelium by using the M cells [9]. M cells inhabited by macrophages which engulf *Shigella*, but instead of successfully destroying it in the phagosome, the macrophage succumbs to apoptotic death [10]. This macrophage cell death is accompanied by the release of the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18. IL-1β signaling sets off the strong intestinal inflammation characteristic of shigellosis.

All these processes consisting of macrophage killing, destruction of the epithelial layer and the massive influx of PMN cells, worsen the bacterial infection and tissue lesion. The severe tissue destruction caused by *Shigella* spp. results in an impaired adsorption of water, nutrients, and solutes, which might cause the watery diarrhea as well as the blood and mucus in stools characteristic of shigellosis [11].

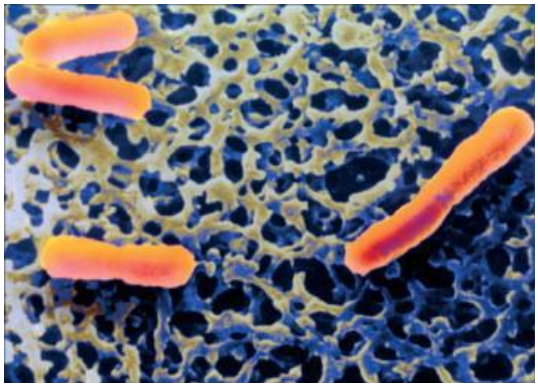

Fig 1: Colored scanning electron micrograph showing *Shigella flexneri* [12]

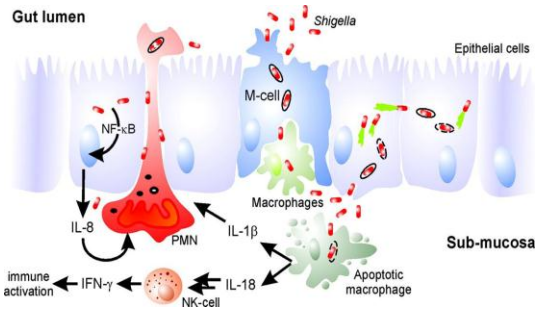

Fig 2: Cellular pathogenesis of *Shigella* spp. [11]

1.2 Immunologic responses against *Shigella flexneri*

Immunologic response against *S. flexneri* is provided by both the innate and the adaptive immune systems. The interactions

between *Shigella* and adaptive immune system, such as T and B lymphocytes, have not been thoroughly investigated due to the lack of appropriate animal infection models that mimic human intestinal infection [13].

Human serum is considered an important host defense mechanism against invasive diseases caused by gram negative bacteria. The complement system, which exists in the blood, is a vital component of the immune system. The system consists of at least 30 proteins that orchestrate attack on pathogenic agents [14]. It has been reported that complement protein is essential for killing susceptible gram-negative bacteria [15]. The functions of complement are numerous but it is most well-known for its capacity to kill pathogens by creating pores in their surface membranes. Complement also participates in inflammatory reactions by attracting phagocytic cells to the site of injury. By opsonising pathogens, complement proteins can stimulate phagocytosis, a process that is mediated by complement receptors on the surface of phagocytic cells [16].

The main barriers that control *Shigella* infection are neutrophils and monocytes. In response to inflammatory stimuli, neutrophils migrate from the circulating blood to infected tissues, where they efficiently bind, engulf and inactivate bacteria. Monocytes arrive to the site of infection within a few hours of *Shigella* infection. It is known that efficient bacterial phagocytosis by monocytes is opsonin dependent. Complement-dependent opsonization of *S. flexneri* with human serum resulted in efficient uptake, following which bacteria did not escape from the phagocytic vacuole and were rapidly killed. Complement-dependent uptake of *Shigella* by both monocytes and neutrophils is therefore likely to be important during the early stages of infection, prior to the production of specific antibody [17].

1.3 Objectives of the Study

The population of Bangladesh, which consists of different groups due to their different lifestyle, food habit, health condition, may all be exposed to this pathogen, *Shigella flexneri*, at one time or other. Human blood serum from these various groups has the ability of exhibiting complement activity against this organism. Serum is the first body defense met by pathogen when it breaks through and enters the skin or epithelial lining of the organs. It contains complement, which can exhibit bactericidal activity through the classical or alternative pathway.

The objective of this study is to compare and investigate the complement activity of human blood serum, collected from urban and slum based population, against *Shigella flexneri*. The lifestyle of people living in urban areas and slum areas are quite different. They do not have similar facilities and hygiene conditions. The population living in slum areas often lacks these and is therefore expected to be more exposed to the pathogen, *S. flexneri*. This comparative study will help to analyze whether the susceptibility of *S. flexneri*, to complement activity of human blood serum, is affected by this lifestyle difference in the urban and slum based population of Bangladesh. This type of work is rare in Bangladesh and therefore it will improve our understanding about the exposure of various population groups to this microorganism and their response against it.

2. Materials and methods

The research study was carried out in the Microbiology Specialized Research Laboratory of the Department of Mathematics and Natural Sciences, BRAC University, Bangladesh.

A strain of *Shigella flexneri* was obtained from the Microbiology Specialized Research Laboratory of the Department of Mathematics and Natural Sciences, BRAC University, Bangladesh.

The serum samples used in the study were collected from two different locations:

i) 50 serum samples were collected from BRAC University, Mohakhali, Dhaka.
ii) 50 serum samples were collected from TNT slum, Mohakhali, Dhaka.

2.1 Procedure for Collection

Blood samples were collected from individuals present at the two study locations, with the help of a trained nurse. Each individual was required to fill up a questionnaire. It contained useful information essential for conducting the study.

Blood sample, about 5ml, was collected by venipuncture procedure, with a sterile disposable syringe and placed into a sterile test tube. Sera from blood sample is separated following standard sera collection technique and the collected serum samples were stored at -20ºC until further use. Repeated freezing and thawing of the samples were avoided in order to ensure better preservation.

2.2 Bactericidal Assay of Human Serum against *S. flexneri*

The bactericidal assay of human serum against *S. flexneri* was carried out by modifying and following some steps of the method used by Bugla-Ploskonska *et al.*[18]

The strains were grown overnight in YP medium and then 50µl of the bacterial culture was transferred to 3 ml of fresh YP medium and incubated at 37ºC for 1h in a water bath. After incubation, the bacterial cells were centrifuged (2500Xg for 20min at 4ºC) and suspended in physiological saline to obtain a six-fold dilution. The bacteria with serum were incubated in a water bath at 37ºC. After 0 (T0) and 180 (T3) minutes, the samples were collected, diluted and cultured on nutrient agar plates for 18 h at 37ºC. The microorganisms were distributed by L-shaped glass after appropriate dilution prior to overnight incubation in 37ºC.

3. Results

The ability of human serum, collected from urban population and slum population, to inhibit growth of *Shigella flexneri* was assessed by taking colony counts from nutrient agar plates at 0 minute and 180 minutes. The plates displayed growth of the bacteria immediately after mixing with serum, at 0 minute and 3 hours after incubation with serum, at 180 minutes.

The following selected figures (Fig 3) display the growth of *S. flexneri* at 0 minute and 180 minutes in 10 representative serum samples collected from urban area (designated as SU1…SUn)

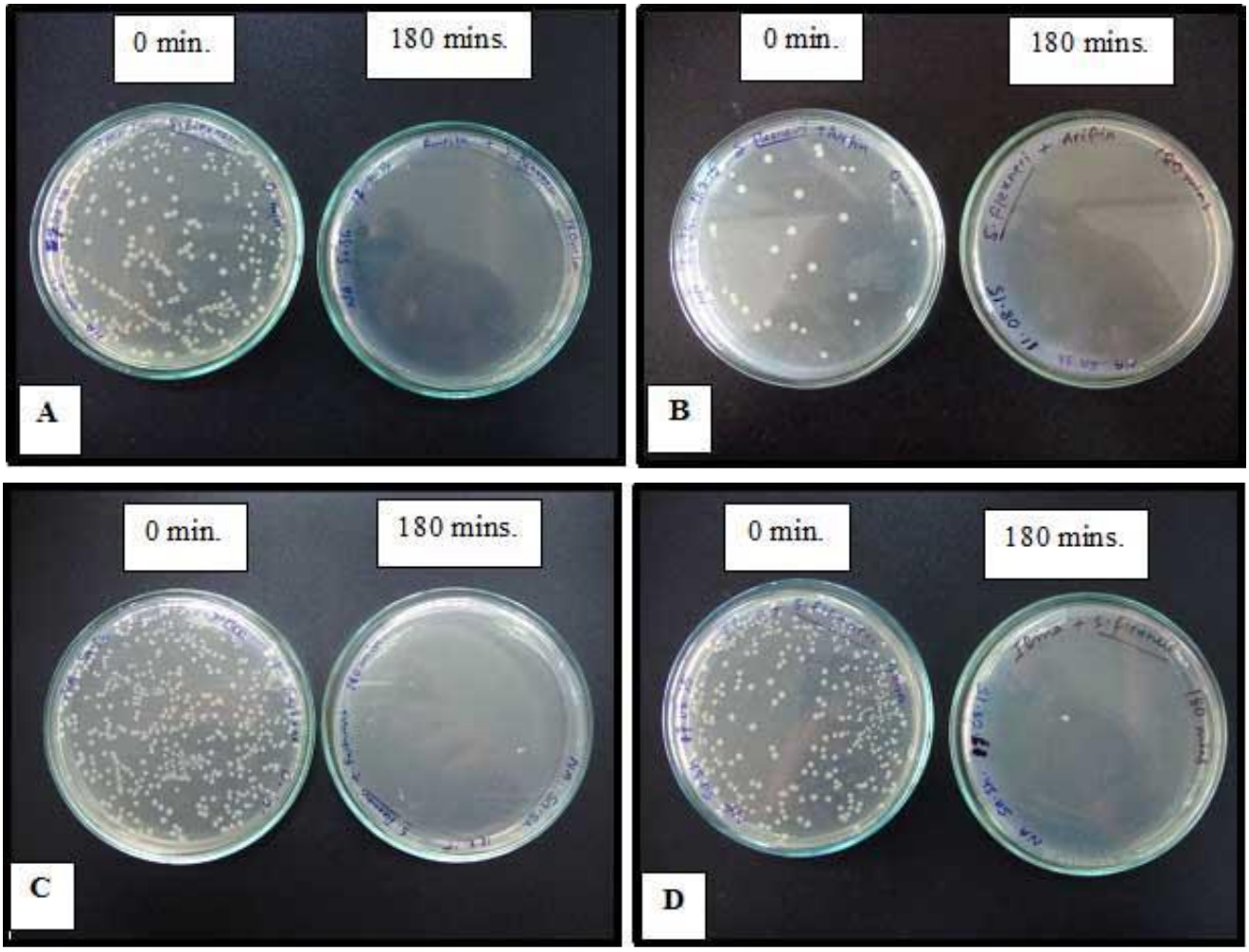

Fig 3: Colonies of *S. flexneri* on nutrient agar at 0 minute and 180 minutes with different serum samples collected from urban population (A) SU1 (B) SU2 (C) SU3 (D) SU4

The following figure (Fig 4) is a graphical representation of serum activity against *S. flexneri* in 10 representative samples of the urban population:

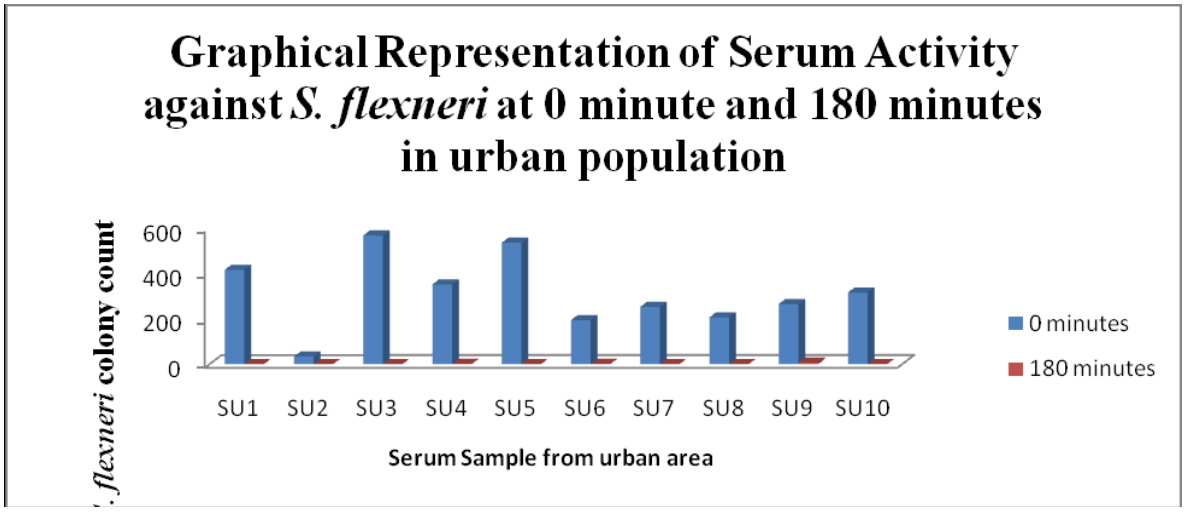

Fig 4: Graphical representation of serum activity against *S. flexneri* at 0 minute and 180 minutes in urban population

The following selected figures (Fig 5) display the growth of *S. flexneri* at 0 minute and 180 minutes in 10 representative serum samples collected from slum area (designated as SS1…SSn):

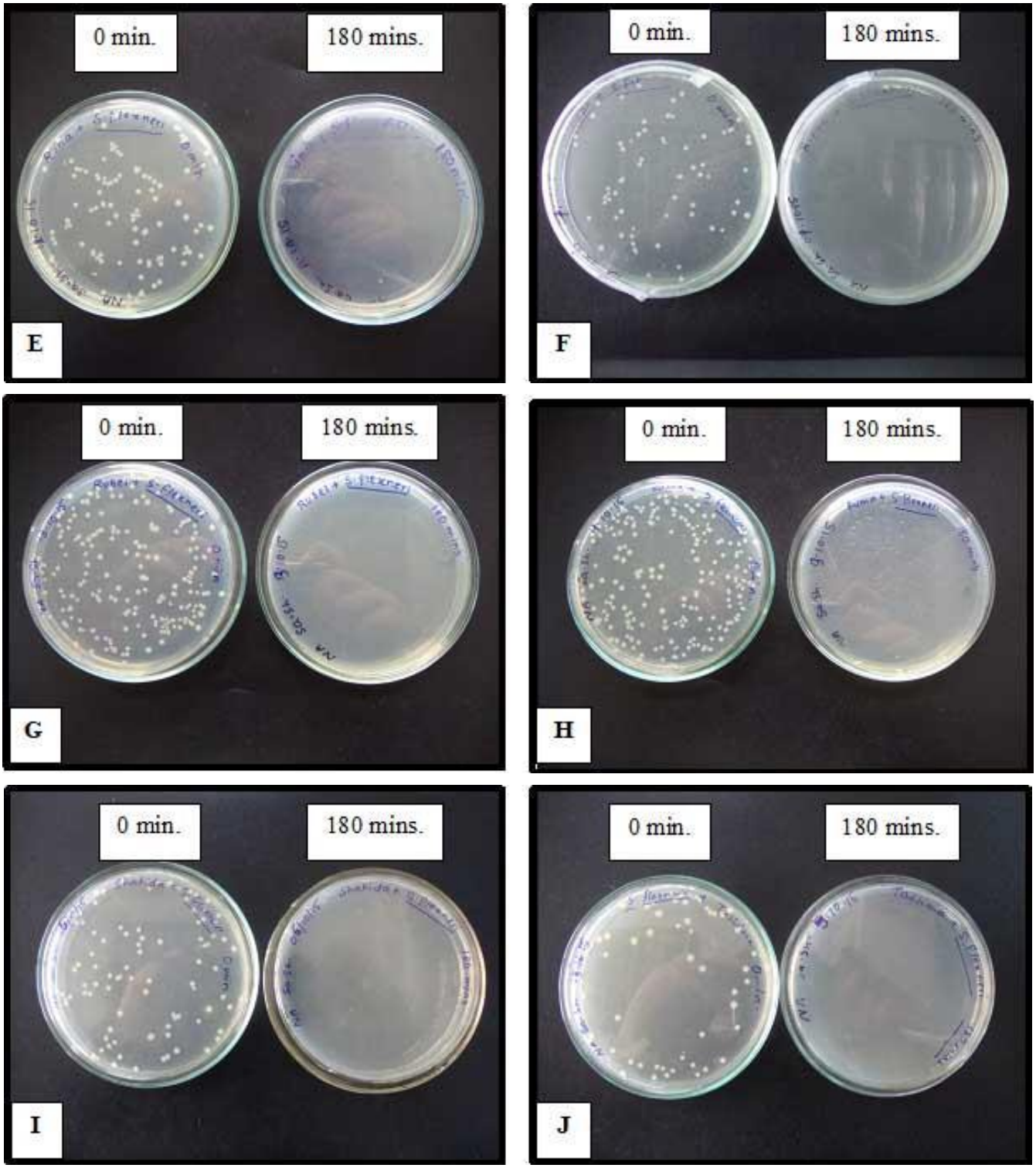

Fig 5: Colonies of *S. flexneri* on nutrient agar at 0 minute and 180 minutes with different serum samples collected from slum population (E) SS5 (F) SS6 (G) SS7 (H) SS8 (I) SS9 (J) SS10

The following figure (Fig 6) is a graphical representation of serum activity against *S. flexneri* in 10 representative samples of the slum population:

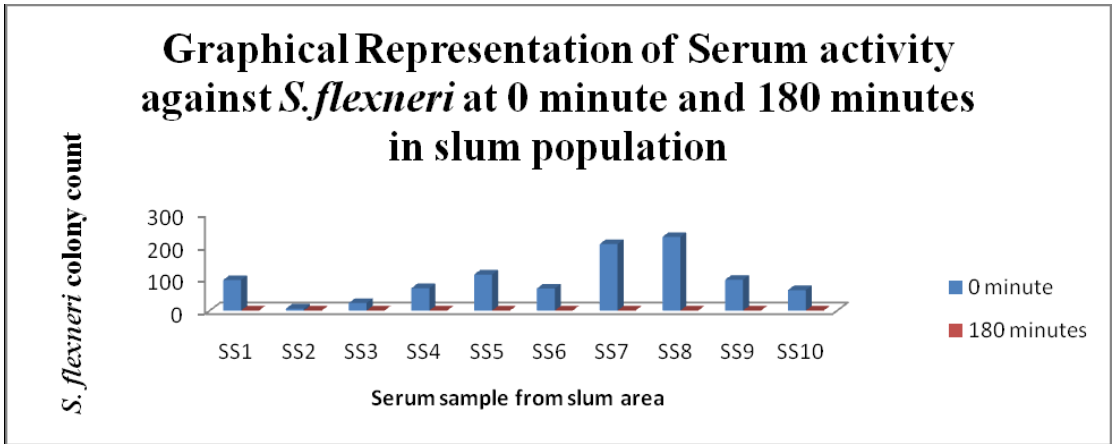

Fig 6: Graphical representation of serum activity against *S. flexneri* at 0 minute and 180 minutes in slum population

In order to compare the results, the average serum activity against *S. flexneri* in different areas were calculated at 0 minute and 180 minutes and depicted in a graph**.**

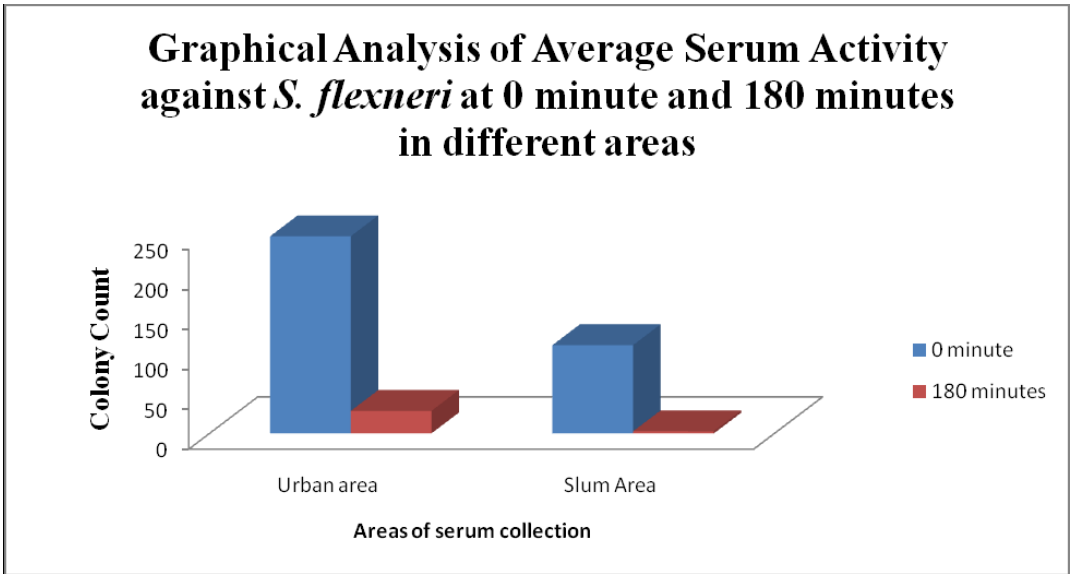

Fig 7: Graphical analysis of average serum activity against *S. flexneri* at 0 minute and 180 minutes in different areas

The percentage inhibition in growth of *S. flexneri* caused by serum samples collected from different areas was calculated for better understanding and comparison of the results.

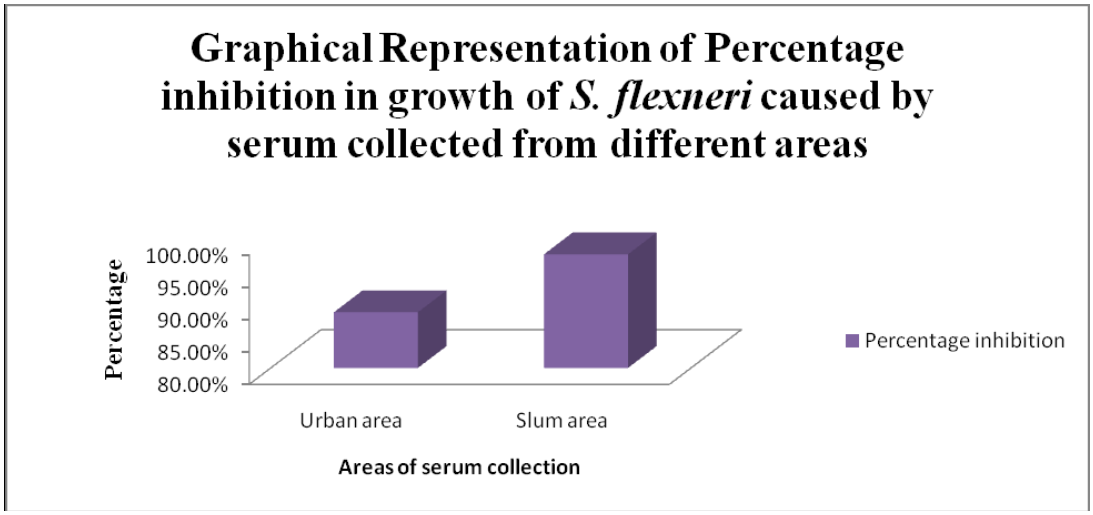

Fig 8: Graphical representation of percentage inhibition in growth of *S. flexneri* caused by serum collected from different areas

4. Discussion

Human serum is considered an important host defence mechanism against invasive diseases caused by gram negative bacteria [15]. It provides the host with antibodies and complement [19]. The major role of the complement system is to recognize and promote the clearance of invading microorganisms [18]. This involves a number of mechanisms including cell-independent bactericidal activity with formation of membrane attack complex, and opsonization for uptake and killing by phagocytic cells [20]. The complement mediated activity of serum might differ in different population groups. Therefore it is important to further investigate this activity and learn more about it.

This study is reporting the comparative complement activity of blood serum against *Shigella flexneri* in urban and slum population. The two population groups are considered due to their significant differences in lifestyle. The slum based population live in overcrowded condition and often lack access to basic sanitation and safe drinking water. This might cause them to have a high exposure to the pathogen *S. flexneri* and exhibit higher resistance against it compared to the urban population.

The results obtained showed that serum samples collected from both urban and slum based population is able to decrease and inhibit the growth of *S. flexneri*. The serum activity against *S.flexneri* at 0 minutes and 180 minutes for 10 representative serum samples collected from urban area and slum area shown in figures. Each sample, from both areas, demonstrates a significant decrease in growth of *S. flexneri* from 0 minute to 180 minutes.

In order to compare the complement activity of serum collected from the different population against *S.flexneri*, the average serum activity was determined. It was observed that the growth of *S. flexneri* at both 0 minute and 180 minutes was higher for serum samples collected from urban area compared to that collected from slum area. The percentage inhibition in growth of *S. flexneri* caused by serum samples collected from these different populations was then assessed. It clearly showed that serum collected from the slum population caused higher inhibition of *S. flexneri* in contrast to that collected from urban population.

5. Conclusion

The results of the study help us to draw the conclusion that the urban population has a better lifestyle in terms of living condition, sanitation, safe drinking water etc. They do not have high exposure to a pathogen like *S. flexneri*, which is not a normal microbial flora of the human body like *E. coli*, and the serum collected from them is unable to inhibit growth of *S. flexneri* as effectively as slum population. The slum population, on the other hand, has repeated exposure to this pathogen and their serum can inhibit its growth more efficiently and exhibit resistance against it.

Further studies are required using diverse population groups to compare the complement activity of serum against *S.flexneri* and find whether lifestyle changes cause any significant difference or not.

6. References

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