International Journal of Engineering & Technology, 7 (2.32) (2018) 513-518

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Comparative Study of Bactericidal Activity of Blood Serum against *Escherichia Coli* in Urban and Slum Population of Bangladesh

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Abstract

Bangladesh is a high densely populated country. Due to the lack of socio economic development it is exposed to lots of water-borne diseases. Of them, *Escherichia coli* or *E.coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of humans and can cause Bloody Diarrhea. This study will help us to analyze whether the exposure of *E.coli*, may accompaniment the activity of human blood serum, which is affected by the socio economic difference in the privileged and under privileged population of Bangladesh. The lifestyle of these two groups are quite different. They do not have similar facilities (in terms of balanced nutritional availability and standard vaccination plan) and hygiene conditions. The population living in slum areas, often privations these and hence expected to be more exposed to the pathogen *E.coli*. The design of this experiment is based on regression analysis by varying serum activity against *E.coli* at 0 minute (initial time) and at 180 minutes, observed in benefited and slum population of urban areas, which has been checked for adequacy and significance via Excel. It will improve our thought about the exposure of various population groups to this bacteria and their response against it. However the under privileged group showed more effective complement mediated killing in comparison to privileged population.

Keywords: *Blood serum, Urban, Rural, Regression analysis.*

1. Introduction

Escherichia coli or *E. coli* is a widely known species of bacteria from the family of gram negative bacteria- Enterobacteriaceae and genus- Escherichia. The genus was named after, German bacteriologist Theodor Escherich who discovered the organism from human colon in 1885. [Fratamico P et al., 2002] Escherich was also the first to show that this organism has the ability to cause certain diseases like infant diarrhea, gastroenteritis etc. The organism was initially named Bacterium coli but later was changed into *Escherichia coli* to honor its discoverer. [Fratamico P P. et al., 2002] It is a gram-negative, facultatively anaerobic, rod shaped bacterium found most commonly in lower intestines of warm blooded animals. The "O" and "H" antigens on the bacteria and their flagella form different serotypes. Though most kinds of *E. coli* do not cause disease in humans, some serotypes have the potential to cause infections of the gastrointestinal tract, urinary tract etc. of their hosts. [Eisenstein et al., 2000] *E. coli* is the major cause of some mild and severe diarrhea. [Bower *et al.*, 1999] The minor infections and severe diseases occur when the pathogenic strain of the bacterium (for e.g. *E. coli* O157:H7) enters host through fecal-oral route. *E. coli* O157:H7 has proved to be one of the most dangerous strains that have a high prevalence of illnesses throughout the world. This strain produces a toxin called shiga toxin that is similar to the toxin produced by *Shigella dysenteriae*. The toxin has the ability to inhibit protein synthesis which leads to the death of cells. This killing of cells leads to a breakdown of the lining and to hemorrhage. The first response is commonly a bloody diarrhea. The toxin also disrupts small blood vessels, mainly the glomerulus which leads to kidney failure and the development of the often hemolytic uremic syndrome. The toxin has effect on lungs and as well as the nervous system. Shiga toxin–producing *E. coli* (STEC) cause approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States. [Mead, et al., 1999 & CDC, 2009] .*E. coli* related enteric infections have a greater prevalence in Bangladesh than most diseases. 34% of the diarrhea related infections are caused by the diarrheal *E. coli* in Bangladesh. People from every background are exposed to this organism by means of environment since *E. coli* is also a beneficial organism inhabiting human and animal guts which occasionally comes in contact with the environment through fecal contamination. But a certain infectious dose is required to develop an infection. When an individual is exposed to the bacteria, different components of blood come forward to play their roles. Complement, as a vital part of the body's immune system, provides a highly effective means for the destruction of the invading bacteria and for immune complex elimination. Since all these antibacterial and antimicrobial components are part of the serum, serum has antibacterial activity. As a developing country, Bangladesh still has a large portion of her people living in slums, below the poverty line. These people have a completely different life style from people living in the urban area. Their living pattern, food habit, hygienity, vaccination etc differs from those who are well off. The objective of this study is to observe and compare the antibacterial capability of human blood serum collected from these two different population groups of two different standards of

living against *E. coli*. A comparative data table according to the result would demonstrate the comparison which would be the basis of the observation from experiment where the factors will be analyzed and comparative bactericidal potential of blood of the two different population of Bangladesh would be documented. Detailed comparative studies like comparing the susceptibility of the people living in slum to people living in urban area against a specific microorganism mediated disease has not been done yet in Bangladesh. So this study has the potential to demonstrate if parameters based on life style, affect the bactericidal activity of serum.

In this research, we have used regression analysis, a statistical technique which helps to explore and model the relationship of Serum activity against *E.coli* at 0 minute and at 180 minutes consecutively observed in urban and slum population. Regression analysis forms an important part of the statistical analysis of the data attained from designed experiments and is discussed briefly in this experiment. Every experiment analyzed in Excel includes regression results for each of the responses.

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 *Escherichia coli* 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 Bactericidal assay of human serum against *Escherichia coli*

Serum is the clear, yellow colored aqueous layer that can be isolated from clotted blood. It contains proteins of different types which participate in the defense mechanism of the body, called complement system. Serum is an environment in which bacterial cells should not exist. The serum complement system provides innate defense against microbial infections. It consists of atleast 35 proteins, mostly in pre-activated enzymatic forms. [Bugla-Ploskońska, G. et al., 2009]. Some steps of the method used in a research paper titled, "Killing of Gram-Negative Bacteria with Normal Human Serum and Normal Bovine Serum: Use of Lysozyme and Complement Proteins in the Death of *Salmonella* Strains O48" were followed in this procedure. [Bugla-Ploskońska, G. et al., 2009] . Each and every work of serum collection was done with extra caution to eliminate unwanted contamination and errors.

3. Results

Identification of the bacteria was done for reconfirmation. *E. coli* was reconfirmed by streaking on a selective media and by performing biochemical tests (Figure 3.1).

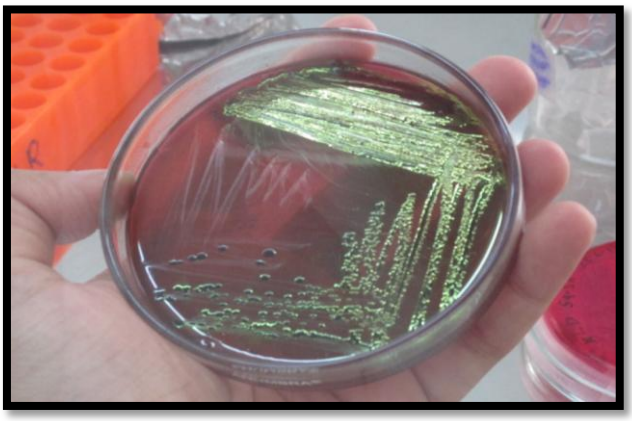

Fig. 3.1: Green sheen *E. coli* colonies on EMB

Table 3.1: the ability of representative serum samples from urban and slum area (BRAC University), to inhibit the growth of *E. coli* at 0 minute

Sample No.	0 min (Urban Population)	0 Min (Slum Population)
1	31	1
2	90	39
3	51	6
4	212	63
5	175	130
6	13	95
7	157	1
8	191	23
9	18	5
10	43	94
11	30	252
12	206	47
13	121	350
14	136	68
15	28	46
16	399	111
17	279	69
18	500	195
19	302	44
20	310	10
21	96	85
22	396	205
23	131	138
24	144	173
25	80	227

3.1 Serum activity analysis

The following table (Table 3.1 and 3.2)and relevant diagrams (Figure 3.2, and 3.3)show the activity of serums collected from both urban and slum area, against *E. coli* at two different times. The 0 minute result usually shows a high count due to the lack of time which the serum needs to act upon the bacteria. But with time as the serum starts to inhibit the growth of the bacteria, the 180 minutes result shows a low count.

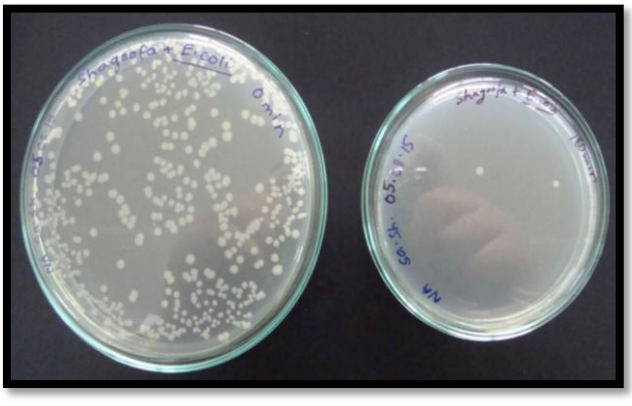

Fig. 3.2: Serum Samples from Urban Area

Table 3.2: The ability of representative serum samples from urban and slum area (BRAC University), to inhibit the growth of *E. coli* at 180 minutes

Sample No.	180 min (Urban Population)	180 Min (Slum Population)
1	0	0
2	0	0
3	1	0
4	0	0
5	3	27
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	10	9
12	0	31
13	4	0
14	0	0
15	0	1
16	0	0
17	3	0
18	4	310
19	325	0
20	366	0
21	7	0
22	426	0
23	6	0
24	0	0
25	24	0

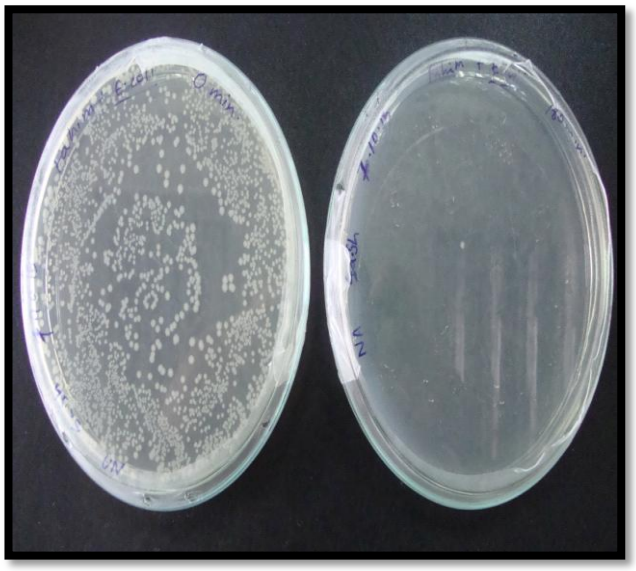

Fig. 3.3: Serum Samples from Slum Area

3.2 Statistical Analysis

The statistical analysis was performed using the Regression analysis test which is used to describe relationships among variables. Overall, this survey serves the purpose of aiding, strengthening and supporting the research procedure and thus establishing an organized, data based result.

3.3 Data analysis of the serum activity of bloody diarrhea causin *E. coli* at 0 minute

Referred to data Table 3.1 and Figure 3.4 and 3.5, R square equals to 0.184. It is not a very good fit. Approximately 18% of the variation in *E.coli* disease detection during the third hour of observation (180 minute later) between urban and slum area has been successfully identified.

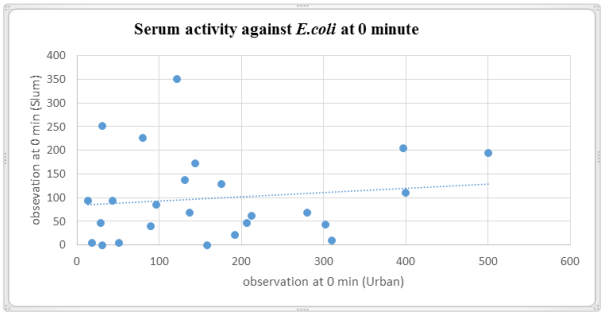

Fig. 3.4: Serum activity against *E.coli* at 0 minute observed in urban and slum population

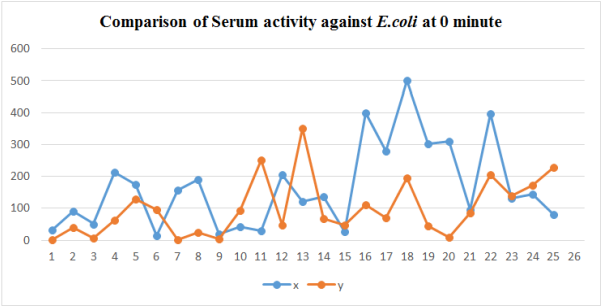

Fig. 3.5: Comparison of Serum activity against *E.coli* at 0 minute observed in urban and slum population

Summary output

Regression Statistics	
Multiple R	0.506592439
R Square	0.256635899
Adjusted R Square	0.185839318
Standard Error	6.380284796
Observations	24

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	295.1313	147.5656	3.624976	0.044423895
Residual	21	854.8687	40.70803		
Total	23	1150			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	7.616848289	2.552157	2.984475	0.007068	2.309347748	12.92434883	2.30934775	12.92434883
Urban	0.017589417	0.01002	1.755363	0.093776	-0.003249118	0.038427951	-0.0032491	0.038427951
Slum	0.027842616	0.014801	1.881171	0.073887	-0.002937085	0.058622317	-0.0029371	0.058622317

Our significance *F* is less than 0.11. Therefore the result is reliable or we can say it is statistically significant. While observing the P-values 0.007, 0.938, and 0.073. The value 0.007 is way below than 0.05, and hence it is significant. The value 0.073 is above 0.05 but close to 0.05, so it is only slightly not significant. The value 0.938 is so much higher than 0.05, and hence not significant.

Residual output

Observation	*Predicted Y*	*Residuals*
1	11.55984641	-10.5598
2	11.55984641	-9.55985
3	11.58449871	-8.5845
4	11.55984641	-7.55985
5	12.12943054	-7.12943
6	11.55984641	-5.55985
7	11.55984641	-4.55985
8	11.55984641	-3.55985
9	11.55984641	-2.55985
10	11.55984641	-1.55985
11	11.97157846	-0.97158
12	12.12889991	-0.1289
13	11.6584556	1.341544
14	11.55984641	2.440154
15	11.57820298	3.421797

16	11.55984641	4.440154
17	11.6338033	5.366197
18	17.34899055	0.651009
19	19.571843	-0.57184
20	20.58258719	-0.58259
21	11.73241249	9.267588
22	22.06172502	-0.06173
23	11.70776019	11.29224
24	11.55984641	12.44015
25	12.15150154	12.8485

3.4 Data analysis of the serum activiy of bloody diarrhea causin *E. coli* at 180 minute

Referred to data Table 3.2 and Figure 3.6 and 3.**7,** R square equals to 0.184. It is not a very good fit. Approximately 18% of the variation in E.coli disease detection during the third hour of observation (180 minute later) between urban and slum area has been successfully identified.

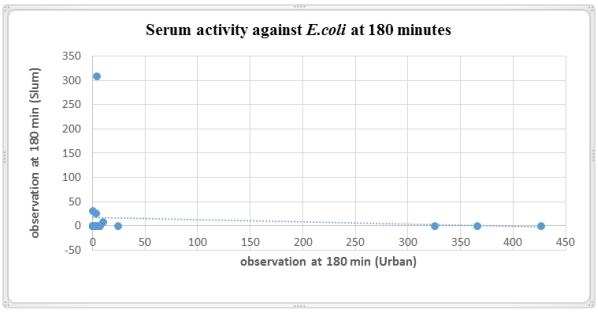

Fig. 3.6: Serum activity against *E.coli* at 180 minutes observed in urban and slum population

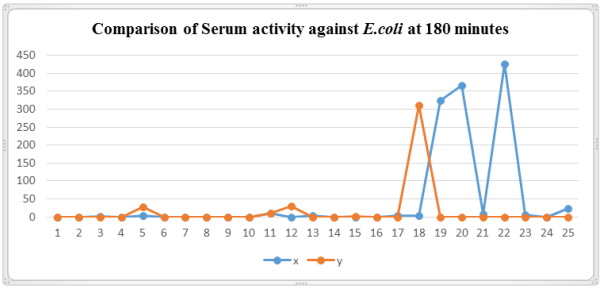

Fig. 3.7: Comparison of Serum activity against *E.coli* at 180 minutes observed in urban and slum population

Summary output

Regression Statistics	
Multiple R	0.428584565
R Square	0.183684729
Adjusted R Square	0.10947425
Standard Error	6.945272597
Observations	25

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2	238.7901	119.3951	2.475185871	0.107259836
Residual	22	1061.21	48.23681		
Total	24	1300			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	11.55984641	1.542428	7.494577	1.714E-07	8.361046217	14.7586466	8.361046217	14.7586466
0	0.024652297	0.011522	2.139571	0.043734513	0.000756976	0.048547618	0.000756976	0.048547618
0	0.018356564	0.02297	0.799144	0.432750761	-0.029280903	0.065994031	-0.029280903	0.065994031

Our *significance F* is less than 0.11. Therefore the result is reliable or we can say it is statistically significant.

While observing the P-values 0.007, 0.938, and 0.073. The value 0.007 is way below than 0.05, and hence it is significant. The value 0.073 is above 0.05 but close to 0.05, so it is only slightly not significant. The value 0.938 is so much higher than 0.05, and hence not significant.

Resıdual output

Observation	*Predicted Y*	*Residuals*
1	11.55984641	-10.5598
2	11.55984641	-9.55985
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4. Conclusion:

Escherichia coli approaches humans with various diseases, despite it is being a beneficial instinctive vegetation. *E. coli* has proved to be one of the major causes of mild and severe diarrhea [Bower *et al.*, 1999]. Since the organism is adapted to flourish in the intestinal environment, the incidence of intestinal diseases caused by the species is higher than that of others. Gradually, *E. coli* has been able to expand its habitat in the human body and has been able to cause diseases like urinary tract infections, bacteremia, cholangitis, pneumonia, neonatal meningitis etc. [Madappa, T., *et al*., 2015].

As *E. coli* is a normal flora of the intestines of animals, the organism is found abundantly in the environment through feces. So each and every individual comes in contact with it. Intestinal diseases with higher prevalence than other *E. coli* related diseases, occur when the organism enters human body through fecal contaminated food, water etc. *E. coli* related enteric infections have a greater prevalence in Bangladesh than most diseases. 34% of the diarrhea related infections are caused by the diarrheal *E. coli* in Bangladesh. Bangladesh being a densely populated, small developing country, a large portion of the population is living below the poverty line. Naturally this part of the population is more exposed to the organism than those who are well off. It can be assumed that people who are living in slums would be more adapted to *E. coli* than those who do not. This study is reporting if the different lifestyle of the two sections of the population (one living in urban area, the other in slum area) of Bangladesh has any effect on the complement inhibition activity of the serums collected from both the places against *E. coli*. The comparative complement activity can be obtained from the column charts from the result section.

In the analysis of the average complement activity against *E. coli* at 0 Minute and 180 Minutes among urban and slum area, the average of the cell counts of 50 serum samples, both at 0 minute and 180 minutes was taken. This was done for both urban and

slum area samples. According to the F-test the result is statistically significant at 0 minute and at 180 minutes.

The differences in living condition or life style do affect the complement arbitrated inhibition activity of human serum. Both the urban and slum population more or less has been exposed to *E. coli* but the slum population has adapted to the organism since they are more exposed to it through poor living condition. The complement system of their serum has been able to destroy more of the organism. Thus the slum residents are able to show greater resistance than that of urban against *E. coli*. The residents of slums can be more exposed to various potentially pathogen organisms due to an underprivileged lifestyle compare to the people who live in the urban area. This exposure may actually aid the slum people in having a better resistance.

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