SOCIOECONOMIC STATUS AND THE GROWTH OF BACTERIA IN HUMAN SERUM

by

Emily K. Corrigan

Submitted in partial fulfillment of the

Requirements for Departmental Honors in

The Department of Psychology

Texas Christian University

Fort Worth, Texas

May 6, 2019

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OF BACTERIA IN HUMAN SERUM

Project Approved:

Supervising Professor: Sarah Hill, Ph.D.

Department of Psychology

Gary Boehm, Ph.D.

Department of Psychology

Shauna McGillivray, Ph.D.

Department of Biology

ABSTRACT

Individuals with a lower socioeconomic status (SES) have an increased risk of a variety of diseases compared to those with a higher SES. The mechanisms underlying the relationship between SES and health are only beginning to be understood. However, recent research finds that a lower SES may induce changes in the immune system that play an important role in determining one's risk for disease. Building on these insights, I sought to examine whether having a lower SES may also increase one's susceptibility to infection by impacting bacterial growth in serum. I measured participants' SES and grew *Staphylococcus aureus* in their serum *ex vivo* for 24 hrs. I then measured bacterial growth at 10 time points (1–8 hrs, 12 hrs., and 24 hrs.). Results revealed higher *S. aureus* growth in the serum of low SES participants (compared to high SES participants). These findings suggest that having a low SES may increase ones vulnerability to infection by making their blood more hospitable to bacterial growth.

Socioeconomic Status and Growth of Bacteria in Human Serum

Chronic health issues are on the rise within the United States and the increase in disease is not distributed evenly across the population. Individuals with low socioeconomic status (SES) have been noted to have an increased risk of in mortality and morbidity of several chronic diseases (Milaniak & Jaffee, 2019). For example, research has concluded that individuals with low SES exhibit an increased risk for metabolic disorders (Delpierre et al., 2016), cardiovascular disease (Murphy et al., 2017), mental health issues (Mock & Arai, 2011), and even certain infectious illnesses (e.g., Cohen et al., 2008).

Scientists have made significant efforts in the past 10 years to begin to understand the mechanism behind the increased risk of disease that is associated with low SES. A growing amount of evidence points towards chronic low-grade inflammation as one causal factor mediating the relationship (Miller et al., 2011). Normally, when the immune system encounters a pathogen, pro-inflammatory cytokines are released, and the immune system mounts defenses against foreign substances. In normal conditions, this type of response is adaptive and allows organisms to survive in a pathogen-dense world. However, this same type of inflammatory response can quickly become maladaptive when activated in the absence of a pathogen, like in the case of chronic stress (Milaniak & Jaffee, 2019). Individuals with low SES have an increase in overall stress levels (Vliegenthart et al., 2016) and the chronic stress associated with low SES has been found to lead to an increased expression of proinflammatory genes and proinflammatory response (Levine et al., 2015; Miller & Cole, 2012; Miller et al., 2009; Miller & Chen, 2010; Murray et al.,).

Not only does the proinflammatory response increase susceptibility to chronic health issues like metabolic disorders (Delpierre et al., 2016), cardiovascular disease (Murphy et al.,

2017), and mental health issues (Mock & Arai, 2011), but it also decreases an effective immune response. A decreased effective immune response increases susceptibility to infectious disease (Marsland et al., 2002), with low SES individuals even exhibiting an increased risk for developing the common cold (Cohen et al., 2008).

Building on these insights, the current study sought to examine whether SES was related to another marker of potential infection risk by measuring the growth of *Staphylococcus aureus* (*S. aureus*) – a common pathogen (Chiller, Selkin & Murakawa, 2001) – in participants' serum. Specifically, I wanted to test whether the growth rate of *S. aureus* in participants' serum was predicted by their SES.

Previous research has indicated that there are differences in growth rates of bacteria between individuals because of varying levels of glucose, phosphate, or amino acids (Figdor et al. 2003), as well as iron concentration differences (Cross et al. 2015), and presence of pro-inflammatory cytokines (Meduri et al., 1999). Iron concentration varies between socioeconomic groups (Keskin et al., 2005; Taylor et al., 1995) as well as glucose (Robbins et al., 2005), and proinflammatory cytokines (Steptoe et al., 2002). Because there are clear differences in serum composition between socioeconomic groups, it can be expected that there should be differences in growth rate of bacteria. With this in mind, I predicted that *S. aureus* would grow more quickly in the serum of low SES individuals (compared to high SES individuals).

Method

Participants

This research was approved as adhering to ethical standards the Texas Christian University Institutional Review Board (Approval #: 1411-117-1606). For this study, participants were recruited using the Texas Christian University Sona System and a written informed consent

was obtained from each participant. The sample included 159 participants who were either students at Texas Christian University or members of the surrounding community. There were 80 males and 79 females ranging from the age of 17-30 (M = 20.17, SD = 2.75). The racial demographic of the sample was as follows: White 66.67% (n = 106), Black 4.40% (n = 7), Hispanic 15.09% (n = 24), Asian 6.29% (n=10), Multiracial/Other 7.55% (n=12). Participants were 1) without a history of chronic medical problems, including depression and other mental illnesses, 2) non-obese (body mass index [BMI] below 30), 3) free from acute illness for the two weeks prior to participation, 4) not on hormonal contraceptives (females), 5) willing to abstain from steroidal and non-steroidal anti-inflammatory medications, exercise, and alcohol consumption for two days prior to participation, and 6) willing to fast the morning of participation. Women participated 4–7 days after the initiation of their most recent menstrual cycle (e.g., early follicular phase).

Materials and Procedure

For these sessions, participants arrived at the laboratory at 7:30 AM, provided informed consent, and were asked additional study compliance questions (e.g., not feeling ill, fasting for eight hours, abstaining from drugs and alcohol). Using the Qualtrics survey program, participants were instructed to complete a series of survey measures (including the target measure of SES). For the purpose of a larger study, additional questionnaires and behavioral tasks were completed before participants had 85mL of blood drawn via venipuncture into heparinized (or EDTA-containing) Vacutainer® tubes (Becton-Dickinson, Franklin Lakes, NJ). Serum was then extracted from the blood samples and stored at -80°C immediately after collection and centrifugation. We later thawed participants' serum and measured growth rates of *S. aureus* in each individual's sample.

Measure of socioeconomic status. To measure participants' socioeconomic status, they completed the McArthur Perception of Wealth Survey. For this task, participants are presented with a 10-step ladder and are told that each rung is a representation of socioeconomic status of their community; the top step, or 10, is the wealthiest in the community while the bottom step, or 1, is the least wealthy in the community. The participants were instructed to choose where they would fall on the ladder ($M = 6.62, SD = 1.63$).

Growth of *Staphylococcus aureus*. To measure growth rates of the human pathogen *S. aureus* (strain Newman) in media supplemented with participant serum *ex vivo*, bacteria were first grown overnight for approximately 18 hours at 37°C in 1mL Lysogeny broth (LB; CulgGenex, Santa Maria, CA) in open air, with continuous shaking. The next day, bacteria were diluted in 3mL LB to an optical density of less than .01 (600nm [OD_{600}]) and grown an additional two hours until reaching the mid-log phase of growth (i.e., OD_{600} of .4), and measured utilizing a spectrophotometer (Spectronic 20D+, Thermo Fisher Scientific, Waltham, MA). Via serial dilution and plating on tryptic soy agar plates, this OD_{600} value was determined to correspond to approximately 7×10^8 colony forming units (CFU)/mL. Next, 1mL of the culture was centrifuged, after which LB was removed and the pellet re-suspended in 1mL RPMI-1640 growth medium. Bacteria were further diluted 1:100 in RPMI-1640 and subsequently plated 1:1 in triplicate with participant serum (200µl final volume) in Falcon® 96-well tissue culture plates (Corning, Corning, NY) and incubated for 24 hrs at 37°C, 5% CO_2, and 100% humidity. Optical density (OD) values were measured every hour for the first eight hours, and then again at 12 hr and 24 hr at a wavelength of 600nm using a plate reader (BMG LabTech FLUOstar™ Omega, Cary, NC). The *S. aureus* growth assay was performed using thawed participant serum that had been frozen at -80°C immediately after collection and centrifugation.

Data Analysis Plan. To test for SES-based differences in the growth of *S. aureus* in participants' serum, we used multilevel growth modeling in MPlus statistical software (version 8). This statistical method controls for dependence in the data, whereby time points were nested within each participant and thus non-independent. In addition, this method allowed me to test whether a continuous predictor (i.e., SES) predicted the continuous outcome of *S. aureus* growth. First, I regressed growth rate on time at level 1, which modeled change in levels of *S. aureus* over time. At level 2, I regressed growth on SES, which modeled whether SES predicted a) overall levels of *S. aureus* across time, b) differences in *S. aureus* levels at each time point, and c) the rate of *S. aureus* growth from hour 1 to hour 24.

Results

Results revealed, first, that *S. aureus* levels increased from hour 1 to hour 24, $\beta = .95$, $SE = .003$, $t = 355.45$, $p < .001$. Across time, SES was negatively relative to overall *S. aureus* levels, $\beta = -.21$, $SE = .10$, $t = -2.13$, $p = .03$. In other words, across the 10 time points, individuals with a lower SES had higher levels of *S. aureus* in their serum compared to individuals with higher SES. Results also revealed that the overall rate of change from hour 1 to hour 24 was not predicted by SES ($p = .86$). However, this appeared to be because individuals with lower SES (compared to higher SES) showed high initial growth, which tapered off by hour 24. Indeed, individuals with lower SES had higher levels of *S. aureus* in their serum at every time point up to 12 hrs of growth (ps $< .05$). By 24 hrs., there were no differences in levels of *S. aureus* between individuals with high and low SES ($p = .10$). Results at each time point are shown in Table 1.

Discussion

Poor health outcomes not only cause distress in the lives of the individuals who are affected but also contribute to the growing cost of American health care. Individuals from low

SES environments are known to have an increased risk of chronic health issues including, but not limited to, cancer (Heidary et al., 2013), obesity (Levine et al., 2011), heart disease (Jones et al., 2009), and mental illness (Torres et al., 2013). Recent research has found that the chronic stress low SES individuals face causes an increased proinflammatory response (Levine et al., 2015; Miller & Cole, 2012; Miller et al., 2009; Miller & Chen, 2010; Murray et al.,), leading to a decrease in function of immune system and other organ systems (Milaniak & Jaffee, 2019). individuals with low SES have been shown to have an increased susceptibility to pathogenic infection, including the common cold (Cohen et al., 2008; Marsland et al., 2002). This study investigated the relationship between bacterial growth in human serum and SES as a way to determine if low SES individuals have an increased risk of bacterial infection or an increased rate of bacterial growth.

After measuring the growth of *S. aureus* in serum every hour for 8 hours and again at hours 12 and 24, it was concluded that low SES individuals had an increase in *S. aureus* growth from hours 1-12. The results of this study suggest that individuals from low socioeconomic status, in addition to having an increased risk of chronic health issues, may also have an increased risk of bacterial infection. This research study adds to the growing body of research investigating the relationship between SES and health outcomes.

Some of the limitations of this study include the population being mainly college-aged students from a higher SES background. In addition, because this study was a correlational study, future research may investigate what component of human serum is causing the different rate of growth in the serum and why this substance varies between SES groups. Possible factors mediating the growth include antibodies, complement proteins, or iron and glucose concentration differences (Cross et al., 2015; Cunnion et al., 2001; Figdor et al. , 2003; McQuillen et al., 1994;

Peterson, 1996). In order to determine if these results have significance in rate of infection between SES groups, future research should also be done to investigate whether or not individuals with low SES report being sick more often than their high SES counterparts.

Also worth mentioning, although at first blush it would seem that higher bacterial growth in the serum of low SES individuals may be universally bad, individuals might actually experience benefits from greater exposure to bacteria. For example, individuals with high SES are more likely to suffer from food allergies and hypersensitivity to the environment because of a hyperactive immune response against pathogens (Bergmann et al., 2000; Lambrecht & Hammad, 2017). Many have hypothesized that this could be due to an overactive immune system as a result of a lack of infection during maturation of the immune system (Yazdanbakhsh et al., 2002). Accordingly, the decreased growth of bacteria in high SES serum could possibly be because of this hyperactive immune response and indicate a greater risk for allergies and autoimmune disorders.

This research study was one of the first of its kind and the results not only contribute to a growing body of research on the relationship between SES and immune function, but also bring up many questions regarding the relationship of environment and health and whether or not an increase in bacterial growth could be beneficial or detrimental to an individual.

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Table 1

Growth of S. aureus in Serum

Hour	1	2	3	4	5	6	7	8	12	24
High SES log(OD_{600})	0.01	0.025	0.04	0.055	0.07	0.085	0.1	0.115	0.175	0.35
Low SES log(OD_{600})	0.025	0.04	0.055	0.07	0.085	0.1	0.115	0.132	0.19	0.37

Note. Log = log-transformed; OD = optical density.

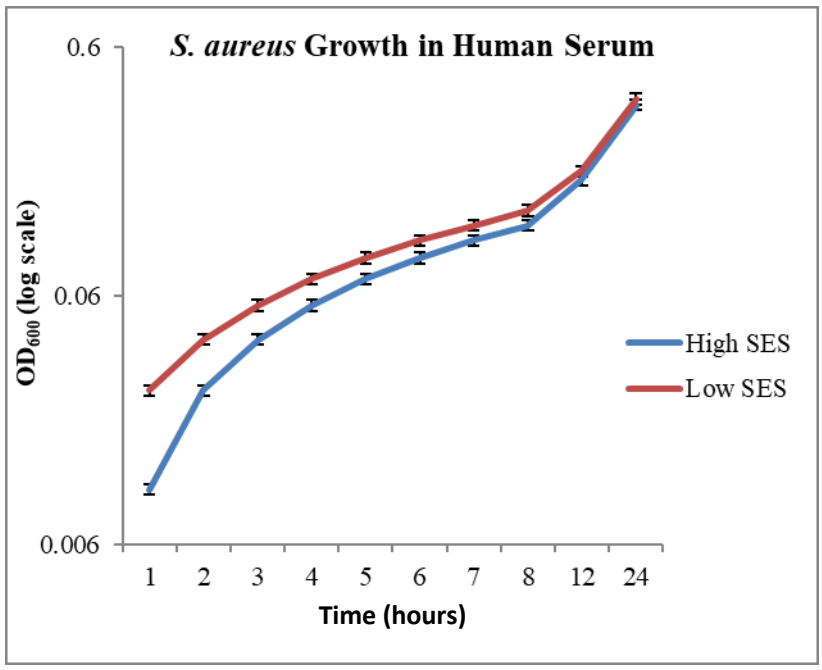

Figure 1. Shown here is *Staphylococcus aureus* (*S. aureus*) growth across time separated by level of socioeconomic status (SES). High SES represents one standard deviation above the mean of SES, while Low SES represents one standard deviation below the mean of SES.