

World Journal of *Clinical Infectious Diseases*

World J Clin Infect Dis 2020 May 15; 10(1): 1-23





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ORIGINAL ARTICLE

Basic Study

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INDEXING/ABSTRACTING

World Journal of Clinical Infectious Diseases is now indexed in China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

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Responsible Electronic Editor: *Ji-Hong Liu*

Proofing Production Department Director: *Yun-Xiaojuan Wu*

Responsible Editorial Office Director: *Ya-Juan Ma*

NAME OF JOURNAL

World Journal of Clinical Infectious Diseases

ISSN

ISSN 2220-3176 (online)

LAUNCH DATE

December 30, 2011

FREQUENCY

Irregular

EDITORS-IN-CHIEF

Joao Mesquita, Caterina Sagnelli, Wei Wang

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2220-3176/editorialboard.htm>

PUBLICATION DATE

May 15, 2020

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<https://www.wjgnet.com/bpg/gerinfo/240>

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<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

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COVID-19 compared to other epidemic coronavirus diseases and the flu

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Author contributions: Ayukekbong JA did the initial literature search, wrote the first draft as well as coordinated the writing of the entire manuscript; Ayukekbong S designed the outline, performed literature search and contributed in writing; Ashu EE, Ntemgwa ML and Agbor TA reviewed the first draft, provided relevant editing as well as writing and performed reference formatting.

Conflict-of-interest statement: The authors declare that they have no competing interests.

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Manuscript source: Unsolicited manuscript

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Abstract

Coronaviruses are among the largest group of known positive - sense RNA viruses with a wide range of animal hosts as reservoir. In the last two decades, newly evolved coronaviruses such as the severe acute respiratory syndrome coronavirus (SARS-CoV) which caused the infamous 2002 outbreak, the Middle East respiratory syndrome coronavirus (MERS-CoV) which caused an outbreak in 2012, and now the SARS-CoV-2 [responsible for the current coronavirus disease 2019 (COVID-19)] have all posed notable threats to global public health. But, how does the current COVID-19 outbreak compare with previous coronaviruses diseases? In this review, we look at the key differences between SARS-CoV, MERS-CoV, and SARS-CoV-2, and examine challenges in determining accurate estimates of the severity of COVID-19. We discuss coronavirus outbreaks in light of key outbreak severity indicators including, disease fatality, pathogen novelty, ease of transmission, geographical range, and outbreak preparedness. Finally, we review clinical trials of emerging treatment modalities and provide recommendations on the control of COVID-19 based on the mode of transmission of the coronaviruses. We also recommend the development and use of a standardized predictive epidemic severity models to inform future epidemic response.

Key words: Severe acute respiratory syndrome, SARS; Middle East respiratory syndrome, MERS; COVID-19; SARS-CoV2; Coronaviruses; Influenza, Flu; Respiratory viruses

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Received: March 17, 2020
Peer-review started: March 17, 2020
First decision: April 3, 2020
Revised: April 15, 2020
Accepted: April 24, 2020
Article in press: April 24, 2020
Published online: May 15, 2020

P-Reviewer: Laassri M, McQuillan GM, Toyoda T, Wang W
S-Editor: Dou Y
L-Editor: A
E-Editor: Liu JH



Core tip: In this review, we look at differences and similarities between severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, and severe acute respiratory syndrome coronavirus 2 and we discuss the challenges in the determination of case fatality rates in pandemics like the current and propose the need for standardization of predictive epidemic severity models that considers critical factors that can influence the severity of outbreaks.

Citation: Ayukekbong JA, Ntemgwa ML, Ayukekbong SA, Ashu EE, Agbor TA. COVID-19 compared to other epidemic coronavirus diseases and the flu. *World J Clin Infect Dis* 2020; 10(1): 1-13
URL: <https://www.wjgnet.com/2220-3176/full/v10/i1/1.htm>
DOI: <https://dx.doi.org/10.5495/wjcid.v10.i1.1>

INTRODUCTION

In December 2019, a cluster of pneumonia was reported in Wuhan, China. Nucleotide sequencing of samples from patients revealed a novel beta coronavirus that was designated novel coronavirus and subsequently as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)^[1,2,3]. The disease caused by this novel coronavirus has been designated by the World Health Organization (WHO) as coronavirus disease 2019 (COVID-19) meaning coronavirus disease of the year 2019. Initial cases of this disease were epidemiologically linked to the Huanan Seafood and Wet Animal Wholesale Market in Wuhan, Hubei Province, China, suggesting a possible zoonotic spill over from wildlife to humans. The disease subsequently spread and the global expansion was facilitated by human to human transmission^[1].

On March 11, the WHO declared the current COVID-19 outbreak, a global pandemic. This is not the first time the world is experiencing a coronavirus epidemic in recent times^[4]. Severe acute respiratory syndrome coronavirus (SARSCoV) occurred in 2002, which reportedly infected 8098 people and caused 774 deaths worldwide. Ten years later, the Middle East respiratory syndrome coronavirus (MERS-CoV) emerged causing a total of 2494 infections, and 858 fatalities^[5]. SARS-CoV-2 is the third coronavirus epidemic to emerge in the human population in the past two decades. Preliminary laboratory investigations suggest that the virus grows in the same cell lines that are used for growing SARS-CoV and MERS-CoV, however, SARS-CoV-2 grows better in primary human airway epithelial cells than in standard tissue culture cells unlike SARS-CoV or MERS-CoV^[6].

But how exactly is COVID-19 different from the SARS or MERS? Initial reports have suggested that the SARS-CoV and MERS-CoV may be more severe than SARS-CoV-2 while the later may be more infectious^[7]. These observations are based on case fatality rates (CFRs), which to the opinion of the authors of this review, is yet to be definitively established for SARS-CoV-2 as we discuss later in this paper. Given how new SARS-CoV-2 is, there are still a lot of unknowns regarding its morbidity and mortality. Since the onset of the disease in late 2019, several important questions regarding the virus and its disease are still being investigated and studied, *e.g.*, what is the shape of the disease pyramid? What proportion of infected people develop the disease? What proportion of infected persons are asymptomatic? And, what proportion of those with the disease die? Furthermore, indices other than fatality and transmissibility are necessary to establish a comprehensive estimate of disease severity. For example, the psychosocial severity of COVID-19 is yet to be determined. Also, because COVID-19 and the flu share commonalities in initial signs and symptoms, questions have been raised on the difference between the two in the context of comparing which epidemic or disease is most serious. However, understanding the differences in seriousness between the COVID-19 pandemic and the seasonal flu needs a comprehensive estimate of epidemic/outbreak severity. Below, we discuss key concepts of epidemic severity including fatality, disease severity, pathogen novelty, preparedness, geographic range, and ease of transmission.

CORONAVIRUS DIVERSITY AND RESERVIORS

Coronaviruses belong to the family *Coronaviridae* which are enveloped, positive-sense, single-stranded RNA viruses of about 80-120nm diameter and 31 kb in size^[8]. There

are at least 7 types of human coronaviruses grouped into either alpha or beta coronaviruses. The alpha coronaviruses include 229E and NL63, and the beta include OC43, HKU1, MERS-CoV, SARS-CoV, and the novel SARS-CoV-2. Acute respiratory infections caused by 229E, NL63, OC43, and HKU1 are often mild while SARS-CoV, MERS-CoV, and SARS-CoV-2 cause both mild and severe disease and have been responsible for global epidemics that began in 2002, 2012, and 2019 respectively^[5]. Coronaviruses are ecologically diverse with the greatest variety seen in bats, which are known to be a reservoir for many emerging viruses. Peri-domestic animals may also serve as intermediate hosts, facilitating transmission to humans. Given the diversity of coronaviruses that infect animals and increasing human-animal interfaces, novel coronaviruses are likely to emerge periodically in humans through cross-species infections and occasional spillover events.

SYMPTOMS AND FATALITY OF SARS-COV-2 AND OTHER EPIDEMIC CORONAVIRUSES

Fatality is the most commonly used indicator to measure disease and outbreak severity. While the CFR is a well-known metric, standardized symptom-scoring metrics for coronaviruses are scarce. Nonetheless, the route of transmission, pathologies, and clinical manifestation of SARS-CoV-2 show resemblance to SARS-CoV and MERS-CoV^[3]. Symptoms of SARS-CoV include fever, cough, dyspnea, and occasionally watery diarrhea. During the epidemic in 2002 - 2003, the virus infected about 8098 individuals resulting in 774 fatalities, placing the CFR at 9.6%^[8]. MERS-CoV on the other hand caused explosive nosocomial transmission events, in some cases linked to a single super spreader. According to the WHO, as of November 2019, a total of 2494 persons had been infected with the MERS-CoV resulting in 858 deaths (CFR of 34.4%) with the majority in Saudi Arabia (Table 1). The clinical features of MERS share many similarities with SARS and COVID-19 such as severe atypical pneumonia, gastrointestinal symptoms, and acute kidney failure^[9]. With regards to the CFR for COVID-19, a recent study by researchers from China's Center for Disease Control and Prevention revealed some interesting clinical features on 44672 confirmed cases that were associated with 1023 fatalities (CFR of 2.3%) (Figure 1). The fatality was significantly higher in older patients (up to 14.8% in patients over 80). In critically ill patients, the death rate was over 49%. Interestingly, the majority of the cases, 81%, were classified as mild, meaning they did not result in pneumonia or resulted in only mild pneumonia, 14% were severe and 5% were critical. More than 87% of cases were aged 30 to 79 years and 2% less than 19 years of age, and 3.8% healthcare personnel were infected.

Finally, it is worth noting that most secondary transmission of SARS and MERS occurred in the hospital settings through super spreaders. Although, the transmission of COVID-19 is occurring in this context too, it appears that considerable transmission is occurring in communities^[10]. Caution should be applied when interpreting these head-to-head CFR comparisons as they might be impacted by confounding independent variables such as time and place.

GLOBAL SPREAD OF SARS-COV, MERS-COV, AND SARS-COV-2

The extent to which an outbreak spreads is dependent on human and environmental factors. Figure 2 shows the geographical distribution of cases of SARS, MERS, and COVID-19 (SARS in 29 countries, MERS in 27 countries, and COVID-19 in 185 countries/regions as of April 12, 2020). Of interest is that, COVID-19 has the largest geographic range. However, it has mostly impacted countries within Asia, Europe, and North America. Africa and South America have experienced the least impact of the coronavirus epidemics in general. Although COVID-19 pandemic has now expanded to these regions (Figure 2), the disease reproduction number is still relatively low. It is not entirely clear why there is limited impact of the disease in Sub Sahara Africa and Latin America, especially considering that transmission may be facilitated by sub-optimal health infrastructure and crowded communities in these regions. On the other hand, it may be construed that the low report of cases may be due to limited testing and surveillance mechanisms. Together, whether environmental factors contribute to the transmission of SARS-COV-2 is obviously an area that requires further research as we learn more about the transmissibility of epidemic coronaviruses. It was recently proposed that high temperature and high relative

Table 1 Comparison of severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus2

	Severe acute respiratory syndrome (SARS-CoV)	Middle East respiratory syndrome (MERS-CoV)	Severe acute respiratory syndrome (SARS-CoV2)
Classification ^[5]	Beta coronavirus	Beta coronavirus	Beta coronavirus
Country of onset ^[4,5,7]	First reported in November 2002 in the Guangdong province, China	First reported in April 2012 in Saudi Arabia	First reported in December 2019 in Wuhan, China
Origin ^[2,4]	From bats, which infected civets and then humans	From dromedary camels to humans	Believed to have spread from contact with bats
Global spread ^[5,8]	29 countries worldwide	27 countries worldwide	185 countries and territories worldwide as of April 12, 2020 (ongoing)
Timeline ^[4,5,7]	Last case in 2004	Last case in 2019	Ongoing (as of May 7, 2020)
Cases and fatalities ^[5,8]	It infected 8098 persons and resulted in 774 deaths	It infected 2494 persons and resulted in 858 deaths	About than 3,836,215 cases and 268,999 deaths as of May 7, 2020
Transmission ^[5]	Droplets/contact In addition, it is possible that these viruses might be spread more broadly through the air (airborne spread) especially during an aerosol generating medical procedures.		
Incubation period ^[5]	Typically, 2-7 d or up to 10-14 d in some cases	2-14 d	1-14 d
Symptoms ^[3]	Fever, non-productive cough, sore throat, headache, myalgia, malaise, shortness of breath, chest pain, vomiting, and pneumonia	Fever, severe acute respiratory illness, cough, and shortness of breath, and pneumonia	Fever, cough, headache, body weakness and myalgia (fatigue), shortness of breath, and breathing difficulties. In severe cases, individuals may show symptoms of pneumonia
CFR ^[4,5,8]	9.6%	34.4%	2.2% (initial reports)
Treatment ^[4]	There are no antiviral drugs effective against coronaviruses. Supportive treatment using corticosteroids (methylprednisolone) to reduce lung injury induced by inflammation has been used to reduced acute respiratory distress		
Vaccines ^[4]	There is no approved and marketed vaccine against SARS-CoV, MERS-CoV, or SARS-CoV-2		

SARS-CoV: Severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; CFR: Case fatality Rate; The ratio of deaths from a disease to the total number of people diagnosed with this disease for a certain period of time.

humidity significantly reduce the transmission of COVID-19. The authors suggested that one-degree Celsius increase in temperature and one percent increase in relative humidity lower R_0 (basic reproductive number) by 0.0383 and 0.0224, respectively^[11]. It is still unclear if this could be a reason for the low transmission in tropical regions. However, this hypothesis may suggest that the arrival of summer and rainy season in the northern hemisphere may affect the transmissibility of the virus. Sociocultural differences in human interactions in different parts of the world may also explain differences in transmission and epidemic expansion; *e.g.*, in contemporary Europe, salutation of friends and close acquaintances is often accompanied by hugging and a kiss on both cheeks. Such close and direct contact with infected persons who may be asymptomatic or unaware of their infections (given the long incubation period of the virus) may facilitate the spread of the virus. Similarly, the coincidence of the onset of COVID-19 outbreak, just prior to China's annual Lunar New Year holiday, was an important factor that had serious impact on the global spread of the disease. Because this is the largest and most important holiday of the year in China, millions of domestic and international trips are made by residents and visitors in often crowded planes, trains, buses, and local transit systems. Therefore, each infected person could have numerous close contacts over a protracted time and across long distances thereby, impacting the global expansion of the disease and complicating response efforts.

PATHOGEN NOVELTY, REPRODUCTION NUMBER, AND THE IMPACT ON TRANSMISSION

The extent to which a pathogen is novel can impact outbreak response, and consequently severity. Factors that determine the extent of pathogen novelty include, knowledge on the pathogen's primary and secondary reservoirs, transmission modes,

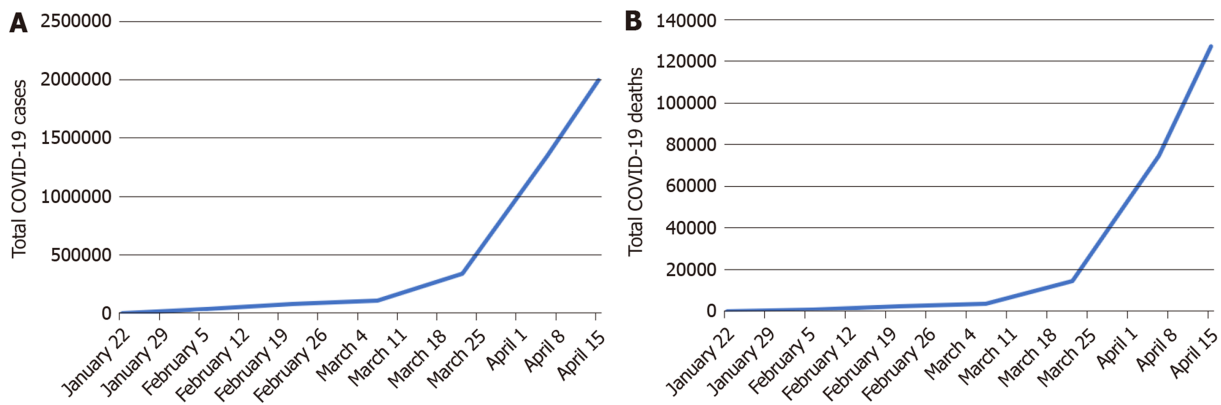


Figure 1 Number of cases of coronavirus disease 2019 and number of deaths due to coronavirus disease 2019 by April 15, 2020. A: Number of cases of coronavirus disease 2019 by April 15, 2020; B: Number of deaths due to coronavirus disease 2019 by April 15, 2020. COVID-19: Coronavirus disease 2019.

control measures, incubation time, diagnostic procedures and treatments, *etc*^[14]. In the case of SARS, the causative agent was only isolated and named after about 5 months into the outbreak, it was absolutely novel at the time^[15]. Unlike this first SARS outbreak, SARS-CoV-2's sequenced genome was already published less than a month after the first case in humans was reported^[15]. Similarly, MERS-CoV was identified at the onset of the outbreak^[14]. Hence it is fair to imply that, SARS was more novel than COVID-19 and MERS when it emerged.

Compared to the MERS outbreak, the SARS and COVID-19 outbreaks showed higher basic reproductive numbers (R_0 ; the expected number of cases directly generated by one case in a population where all individuals are susceptible to infection). The R_0 for SARS and COVID-19 is similar (3 and 3.2 respectively) and MERS is < 1 ^[16-18]. The higher R_0 for SARS and COVID-19 may support the reason why their global spread is higher than MERS.

HOW DOES COVID-19 COMPARE TO THE SEASONAL FLU?

Human coronaviruses such as 229E, NL63, OC43, and HKU1 have long been considered inconsequential pathogens, causing the "common cold" and other mild respiratory symptoms in healthy people^[5,6]. However, in the last two decades highly pathogenic coronaviruses have emerged including the current SARS-CoV-2 causing widespread morbidity and mortality. Although the initial symptoms of both COVID-19 and the flu are associated with acute respiratory infection (Table 2), the global morbidity and mortality of the current COVID-19 pandemic is expected to surpass that of the seasonal flu. So far, the novel SARS-CoV-2 has led to about 3836215 illnesses and 268999 deaths as of May 7, 2020. This fatality is likely to increase before the pandemic resolves. The flu on the other hand sickens about 5 million people worldwide, killing up to 650000 people every year according to the WHO^[19]. Despite these figures, caution should be applied when interpreting global disease burden of these diseases. It is important to note that the burden of infections differ by place (country or region) and time (when). Hence, comparing the CFRs of COVID-19 and the seasonal flu without considering these differences is inappropriate. For example, this season (October 2019 to May 2020) the Centre for Disease Control estimates that as of March 28, 2020, about 24000-63000 of the 39-55 million people who contracted influenza in the United States have died. Historically, the CFR of influenza in the United States has always been $< 0.1\%$. As of May 7, about 76512 of the 1.29M confirmed cases of COVID-19 in the United States had died (CFR = 5.9%). Comparing the CFR of COVID-19 to the CFR of the seasonal flu from earlier years is inappropriate as place and time are independent variables that may influence disease transmission. Finally, it is essential to note that the occurrence of COVID-19 and the flu are not mutually exclusive. COVID-19 could potentially exacerbate the disease burden of the flu and vice versa. Despite the burden of the flu, a lot is known about the virus and the seasonal expectations and projections. In contrast, very little is known about SARS-CoV-2 (which obviously is not a flu), and the outbreak is yet to peak in several countries and jurisdictions. However, so far COVID-19 seems to have spread much faster than the flu causing severe illnesses and leading to a shutdown of the socio-economic activities worldwide. This seems to be a severe disease and its real burden will only be accurately reflected and evaluated post resolution of the

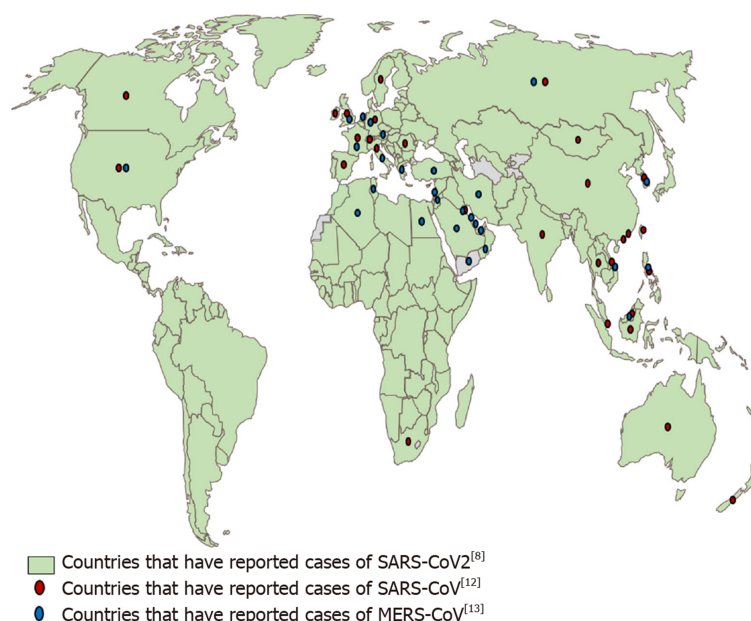


Figure 2 The global spread of severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus2 as of April 15, 2020. SARS: Several acute respiratory syndrome; MERS: Middle East respiratory syndrome; CoV: Coronavirus.

pandemic.

It should be noted that the above comparisons are made against endemic flu. However, the CDC estimates that 151700-575400 people worldwide died from the 2009 H1N1 flu pandemic during the first year the virus circulated. Strangely, > 80% of related deaths were estimated to have occurred in people younger than 65 years of age. This differs greatly from typical seasonal influenza epidemics about 70%-90% of deaths are estimated to occur in people 65 years and older.

THE CASE FATALITY RATIO OF COVID-19, AN UNRESOLVED DILEMMA

The CFR is the ratio of the number of deaths from a disease to the total number of people diagnosed with the disease for a certain period of time. For an emerging infectious disease like the COVID-19, CFR is a vital indicator to assess clinical severity. Initial reports from China and other global health agencies have reported that the CFR of COVID-19 is about 2.2%, relatively lower than SARS or MERS. In Canada and United States, the CFR as of April 6 is 2.2% and 3.4% respectively. However, in Spain and Italy during the same time, the CFR is 10% and 12.6%, respectively. For a disease that is in its nascent stage, we think it is far too early to definitively establish the crude fatality rate of COVID-19. We believe, these initial estimates are based on the intuitive calculation of dividing the death toll by the number of confirmed cases. For example, if we consider the estimate as of April 12, 2020 from Table 1, 113296 deaths divided by 1836338 confirmed cases (No. of deaths + recovered) multiplied by 100, we get a CFR of 6.1% while the CFR as of February 12 was 2.1%. As simplistic as this may be, biases in the estimate of population fatality rates during outbreaks may occur if critical confounding factors are not considered. It has been suggested that CFRs calculated from individual outcome data are likely to be more reliable than estimates calculated from population level data^[23]. If estimates from population-based data are used, they must include the lag time between reporting cases and reporting deaths in order to account for reported cases for whom the disease outcome is yet unknown. This is particularly important if there is a delay from symptom onset to case report or delay from death to fatality report^[24]. Moreover, from previous experience, equal reporting of cases and deaths is unlikely in an emergency pandemic situation, and even less likely to be consistent across multiple countries. This is because different reporting systems may be used by different countries to record confirmed cases and deaths, leading to inaccuracies in estimating the CFR. Simply dividing the total reported deaths by the total reported cases over multiple countries neglects such variability across countries and this may skew the calculation

Table 2 Comparison of coronavirus disease 2019 and the flu

Factors	COVID-19	Flu
Incubation period	2-14 d	1-4 d
Symptoms ^[3]	The most common symptoms are cough, sore throat, headache, body weakness and myalgia (fatigue) due to severe respiratory illnesses associated with shortness of breath and breathing difficulties. In severe cases, individuals may show symptoms of pneumonia	Typical flu symptom is characterized by a sudden onset of fever, cough (usually dry), headache, muscle and joint pain, severe malaise (feeling unwell), sore throat and a runny nose
Case fatality rate ^[8,20]	Initial reports from China suggest the case fatality rate is at least 2.2% (unresolved), and United States 3.9% as of April 12, 2020	The case fatality rate for the flu in the United States is < 0.1%
Virus transmission ^[17,21]	The basic reproduction number, R_0 is about 3.2	The production number of the flu is about 1.28
Characteristic	Pandemic	Endemic, potential for epidemic or pandemic
Prevention ^[19,22]	There is no approved vaccine for COVID-19	There is an annual flu vaccine

COVID-19: Coronavirus disease 2019; R_0 : the average number of people who catch the virus from a single infected person.

of the CFR^[25]. Countries specific variation in CFR is very prominent. As of April 12, 2020, the CFR of Italy was like ten times higher than that of most countries in Africa. Furthermore, the preferential reporting of apparently severe cases or symptomatic infections may neglect mild or asymptomatic infections and this bias can lead to faulty CFR calculation. Unfortunately, monitoring asymptomatic infections in an outbreak situation like the current one is not a public health recommendation and is not an area to prioritize resources during an active pandemic.

Together, as the pandemic spreads rapidly through countries, and as country specific surveillance significantly differ, the CFR estimates may fluctuate substantially. Therefore, without adequate knowledge of the relative reporting of cases to deaths, estimates of CFR calculated from population level data should be interpreted with caution. A retrospective study that will assess the serostatus of close contacts of patients irrespective of symptoms would help to determine the proportion of asymptomatic and mild infections and help guide the calculation of the near true CFR. Until then, the exact reproduction number (R_0) estimate or CFR of COVID-19 still remains an issue to be thoroughly investigated.

OUTBREAK PREPAREDNESS AND THE IMPACT ON DISEASE CONTROL

There is an old adage that says, “luck favours the prepared mind”. Slow and ineffective responses can prolong an outbreak and consequently increase severity. In this section we use the 2019 Global Health Security (GHS) index domains, an outbreak preparedness metric, to explore the outbreak severity of coronavirus outbreaks. GHS is an index that contains 34 indicators organized across 6 domains that measure, (1) Prevention of the emergence or release of pathogens; (2) Early detection and reporting of epidemics of potential international concern; (3) Rapid response to and mitigation of the spread of an epidemic; (4) Sufficiency and robustness of health systems to treat the sick and protect health workers; (5) Commitments to improving national capacity, financing plans to address gaps and adhering to global norms; and (6) Overall risk environment and country vulnerability to biological threats^[26].

Although the GHS index was only recently developed and can't be used for the inferences of prior health events, it is important to note that the frequent emergence and re-emergence of epidemics with pandemic potential has increased outbreak awareness and preparedness in the global community. Between 2011 and 2018, the WHO tracked 1483 epidemics in 172 countries^[26]. The frequency of these outbreaks has led to improved outbreak preparedness globally. A testament to increased outbreak preparedness in the global community is the implementation and monitoring of International Health Regulations (2005), which aim to prevent and control the international spread of disease through committed national leadership, health system strengthening, financing to address gaps, and international collaboration.

The GHS Agenda through its partners in over 64 countries is helping to build capacities to prevent or respond to infectious disease threats. This initiative focuses on 11 areas of action (action packages). Prevent 1: Antimicrobial Resistance; Prevent 2:

Zoonotic Disease; Prevent 3: Biosafety and Biosecurity; Prevent 4: Immunization; Detect 1: National Laboratory System; Detect 2: Real-Time Surveillance; Detect 3: Reporting; Detect 5: Workforce Development; Respond 1: Emergency Operations Centers; Respond 2: Linking Public Health with Law and Multisectoral Rapid Response; Respond 3: Medical Countermeasures and Personnel Deployment Action Package.

Although some countries have achieved significant progress in capacity level improvement in areas like immunization, biosafety, and biosecurity, there is a limited focus on surveillance of zoonotic diseases, infection prevention and control, and early detection capacity of emerging pathogens.

It is also important to mention that the severity of the outbreak can differ significantly by country's readiness. For example, countries with low GHS index are likely to be highly impacted (*e.g.*, the GHS score of the Democratic Republic of Congo is 26.5). If a country such as this is badly hit while already struggling to contain re-emerging Ebola outbreaks, the consequences might be dire. Hence, caution must be applied when inferring the overall severity and impact of the COVID-19 outbreak, as country readiness, capacity level, resources, and context are essential independent variables that should not be neglected in the assessment.

TREATMENT OPTIONS AND CLINICAL TRIALS FOR COVID-19

The emergence of SARS-COV-2 and COVID-19 has left the scientific community searching for potential therapeutics to manage the disease. There is no known effective antiviral against SARS-COV-2, however previously used antivirals and pharmacologics are currently being investigated and in some cases used in the clinical setting on an off-label basis to treat patients suffering from COVID-19. Chloroquine and its hydroxyderivative - hydroxychloroquine, are currently being used to treat COVID-19 patients in some countries across world (*e.g.*, China, France and United States). Chloroquine and hydroxychloroquine have been used for decades for the effective treatment of malaria with a well-known tolerability and safety record. Based on its known *in vitro* antiviral activities against diverse human viruses (reviewed in Devaux *et al*^[27], 2020) and SARS coronaviruses^[27-29], and the recent reports of its *in vitro* efficacy against SARS-COV-2^[30-32], a non-randomized trial to evaluate the clinical efficacy and safety was carried out in small cohort of hospitalized patients with COVID-19 pneumonia in China^[33,34]. Compared to control treatment (Lopinavir/Rotinavir), chloroquine demonstrated superior efficacy in the inhibition of the exacerbation of pneumonia both clinically and based on improved lung imaging findings, shortened disease course and promoted complete viral clearance. In these patients, 500 mg of chloroquine was administered orally twice daily for 10 d. Chloroquine has now been included in the Guidelines for the Prevention, Diagnosis and Treatment of Pneumonia Caused by COVID-19 by the National Health Commission of China^[34,35]. A non-randomized open label trial carried out in France treated hospitalized COVID-19 patients with variable disease severity with a combination of hydroxychloroquine (600 mg/d for 10 d) and azithromycin (500 mg on day one followed by 250 mg/d for four d) or no treatment^[36,37]. Results from this study indicated that hydroxychloroquine and azithromycin were effective treatments for COVID-19 patients resulting in faster clinical improvement and discharge; and complete viral clearance (based on a negative polymerase chain reaction test results or viral culture). Despite the encouraging findings from these studies, it is important to note that the trials were non-randomized, had design flaws with relatively few participants (less than a few hundred participants in each study). It is, therefore, prudent for the scientific community to carry out more well-designed clinical trials to assess the efficacy and safety of chloroquine for the treatment and management of COVID-19 patients prior to making a final recommendation for its use. This will allow for the development of appropriate treatment guidelines including dosage, patient monitoring, duration of treatment and expected outcomes. The United States Food and Drug Administration has since issued an authorization to permit the emergency use of chloroquine phosphate to treat adult and adolescent hospitalized COVID-19 patients for whom a clinical trial is not available, or participation is not feasible^[38]. More than 30 clinical trials are ongoing in different parts of the world on the use of chloroquine for COVID-19 treatment^[39-41]. While chloroquine may be well tolerated, safe and cheap, the drug has a narrow therapeutic index and long-term use may be associated with cardiomyopathy and retinopathy^[42,43]. Toxic concentrations can be lethal as such self-prescription is not recommended and administration should be done only in a hospital setting.

Another drug in clinical trials used for treatment of COVID-19 patients is remdesivir (GS-5734). It is a broad-spectrum antiviral nucleotide analogue with reported efficacy against SARS-CoV1 and MERS-CoV coronaviruses in cell culture and animal models that was used to treat a COVID-19 patient in the United States who showed significant improvement and tolerability one day after intravenous administration of the drug^[32,43,44]. Apart from chloroquine and remdesivir, several drugs both new and old being repurposed for the treatment of COVID-19 are now under clinical trials with the hope that they may be available at patients bed-side in the near future.

The most effective strategy to control the spread, eradicate and minimize the burden of infectious diseases is through mass immunization. Unfortunately, given the novelty of SARS-CoV-2 and COVID-19 and the speed with which the virus spread around the world, scientists have had little time to develop any vaccine candidates. As such there is no known effective vaccine against SARS-CoV-2 at this time, however emerging epidemiological data suggests that the Bacillus Calmette-Guérin (BCG) vaccine (the vaccine for tuberculosis) may be effective in decreasing spread of infection, disease severity and mortality from COVID-19^[45-48]. These reports suggest that there is a correlation between either universal or mandated BCG vaccination and morbidity and mortality from COVID-19. The evidence comes from historical vaccination data review and the current morbidity and mortality rates due to COVID-19 in different countries. Countries without historical universal policies of BCG vaccination at birth such as Italy, Netherlands, United States have been severely afflicted compared to countries with compulsory and long-standing BCG policies consistent with a possible protective role of the BCG vaccine against COVID-19^[45,47]. As promising and hopeful as these data may be, these are epidemiology studies and not controlled trials thus it is imperative for large scale randomized control trials be carried out to test this theory. The BRACE (Australia) and BCG-CORONA (Netherlands) randomized-controlled trials are currently in progress to assess the effectiveness of the BCG vaccine to enhance the immune systems of healthcare workers against COVID-19^[49,50]. Results from these studies will provide empirical data to support the epidemiological reports above and offer some hope to the world. As the pandemic escalates globally, basic infection prevention and control guidelines appear to be the best option to mitigate the spread of the disease.

INFECTION PREVENTION AND CONTROL FOR COVID-19

The route of transmission, pathologies, and manifestation of SARS-CoV-2 clearly show some similarities to SARS-CoV and MERS-CoV. Both SARS-CoV and MERS-CoV infect intrapulmonary epithelial cells better than cells of the upper airways making transmission to occur primarily from patients with recognized illness and not from patients with mild, nonspecific signs^[51]. The incubation period of SARS-CoV-2 is between 1-14 d and patients present with fever associated with flu-like symptoms including cough, sore throat, headache, body weakness and myalgia (fatigue) to severe respiratory illnesses associated with shortness of breath and breathing difficulties^[52]. In critical cases, individuals may show symptoms of pneumonia associated with complications of severe acute respiratory and cardiac distress, and kidney failure, which can eventually lead to death. The long incubation period facilitates the spread of the infection to others through contact and exposure to infected droplets.

It has been suggested that SARS-CoV-2 uses the same cellular receptor (human angiotensin-converting enzyme 2) as SARS-CoV, making transmission to occur mainly after signs of lower respiratory tract disease has developed^[53]. Similar to SARS-CoV and MERS-CoV, the transmission of SARS-CoV-2 occurs by means of droplets and contact with infected persons. Therefore, public health measures and strict adherence to standard precautions in health care settings, are critical in controlling the pandemic^[54]. Together, breaking the chain of transmission of a pandemic like COVID-19 is a shared responsibility; the population and the state have unique roles to play.

Population

Individuals must practice physical distancing (staying 2 metres apart from other people at all times). Anyone who is ill, including mild respiratory symptoms, must stay home and monitor their health for fever, cough or difficulty breathing and based on national legislation, report their symptoms to the public health authorities for tracing and eventual testing. All returning international travellers must stay home for 14 d. The population must be encouraged to practice good hand hygiene and cough etiquette. For example, washing of hands often with soap and warm running water,

or alcohol-based hand sanitizers and covering mouth and nose with the arm when coughing or sneezing to avoid the expulsion of droplets to others. People should avoid touching their eyes, nose, and mouth unless they have just washed their hands. Unnecessary movements should be restricted and if someone should go out for essential visits, he or she should wear a mask that covers the nose and mouth and care should be observed when handling the mask.

Health care establishments

All healthcare establishments should perform active and passive screening. Persons conducting screening should ideally be behind an impermeable barrier to protect them from droplet from sneezing/coughing patients. If a patient screens positive, he or she should immediately be asked to don a surgical mask and be isolated. From this time onwards, healthcare workers should apply standard and transmission-based precautions including the appropriate use of personal protective equipment such as gloves, gown, surgical/procedure masks and eye protection (goggles or face shields) for patient care^[54]. As a general rule, health care workers should use a risk assessment approach before and during each patient interaction to evaluate the likelihood of exposure. In the event that an aerosol generating medical procedure has to be done, droplet, contact and airborne precautions should be observed, and the procedure should be done in an airborne infection isolation room that is under negative pressure. These precautions include wearing the following personal protective equipment - gloves, gown, N95 fit-tested respirators and eye protection (goggles/face shields)^[55,56]. Patients who test positive for SARS-CoV-2 should not be cohorted with non-COVID-19 patients, but may be cohorted with other patients confirmed to have COVID-19. It is essential to routinely clean and disinfect care equipment, surfaces and environment using approved hospital-grade disinfectants.

Governments and public health authorities

It is the responsibility of every nation to protect the lives of its citizens. Once an outbreak of a disease with pandemic potential is determined, there should be declaration of a state of emergency to help contain the spread and protect the public. Consequently, the following establishments are required to closed to prevent congregation of persons; bars and restaurants (except to the extent that such facilities provide takeout and deliveries), indoor recreational centers, public libraries, churches, schools, child care centres, movie cinemas, theatres, concert venues and other communal or shared public or private centres. Additionally, all organized public events of over 5 people (or when a 2 m separation cannot be maintained) should be prohibited, including parades, funeral, weddings, and other social gatherings. As much as possible employees should be encouraged to work from home if feasible. Travel restrictions should be put in place to discourage the population from international travels especially to highly impacted countries. Returning travellers must self-isolate and monitor for symptoms for 14 d.

Also, it is absolutely necessary that the right information is given to the population to avoid the dissemination of false and inaccurate information and all rumours and conspiracies should be debunked with scientific evidence. The population through community leaders should be involved in decision making as an inclusive approach will results in better compliance and positive outcomes.

CONCLUSION

From 2002, there has been a pattern of coronaviruses emerging and causing epidemics every 8-10 years. The SARS-CoV, MERS-CoV, and now SARS-CoV-2 that have been responsible for global epidemics starting in 2002, 2012, and 2019 respectively^[57]. It is known that coronaviruses reside in animal reservoirs but the spillover mechanism into human population is not fully understood. In our opinion, coronaviruses will continue to emerge periodically and unpredictably, spreading and inducing serious infectious diseases of huge global health impact.

Although the first vaccine against COVID-19 is being developed and a chain of therapeutic clinical trials are underway, there are no approved drugs or vaccine for the treatment or prevention of coronavirus infections^[58]. Furthermore, the range of animal reservoirs for coronaviruses makes the threat to the human population worse. A starting point in the prevention of future coronavirus outbreak is the regulation of wildlife meat trades in order to reduce the risk of animal to human spillover of the virus, surveillance and development of laboratory capacities for early detection.

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Basic Study

GadE regulates *fliC* gene transcription and motility in *Escherichia coli*

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Author contributions: Schwan WR, Flohr NL, Multerer AR, and Starkey JC designed the research, performed the research; Schwan WR, Flohr NL, and Multerer AR analyzed the data; Schwan WR wrote the paper.

Supported by the National Institutes of Health, No. 1R15AI-065432.

Institutional review board

statement: No humans or samples from human were used in this study.

Institutional animal care and use

committee statement: No animals were used in this study.

Conflict-of-interest statement: The authors report no conflict of interest.

Data sharing statement: No additional data are available.

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Abstract

BACKGROUND

Escherichia coli (*E. coli*) express flagella to ascend human urinary tracts. To survive in the acidic pH of human urine, *E. coli* uses the glutamate decarboxylase acid response system, which is regulated by the GadE protein.

AIM

To determine if growth in an acidic pH environment affected *fliC* transcription and whether GadE regulated that transcription.

METHODS

A *fliC-lacZ* reporter fusion was created on a single copy number plasmid to assess the effects of acidic pH on *fliC* transcription. Further, a Δ *gadE* mutant strain of a uropathogenic *E. coli* was created and tested for motility compared to the wild-type strain.

RESULTS

Escherichia coli cells carrying the *fliC-lacZ* fusion displayed significantly less *fliC* transcription when grown in an acidic pH medium compared to when grown in a neutral pH medium. Transcription of *fliC* fell further when the *E. coli* was grown in an acidic pH/high osmolarity environment. Since GadE is a critical regulator of one acid response system, *fliC* transcription was tested in a *gadE* mutant strain grown under acidic conditions. Expression of *fliC* was derepressed in the *E. coli gadE* mutant strain grown under acidic conditions compared to that in wild-type bacteria under the same conditions. Furthermore, a *gadE* mutation in a uropathogenic *E. coli* background exhibited significantly greater motility than the wild-type strain following growth in an acidic medium.

CONCLUSION

Together, our results suggest that GadE may down-regulate *fliC* transcription and motility in *E. coli* grown under acidic conditions.

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Manuscript source: Invited Manuscript

Received: February 7, 2020

Peer-review started: February 7, 2020

First decision: March 5, 2020

Revised: March 25, 2020

Accepted: May 5, 2020

Article in press: May 5, 2020

Published online: May 15, 2020

P-Reviewer: García-Elorriaga G, Nagata T, Song G

S-Editor: Wang J

L-Editor: A

E-Editor: Liu JH



Key words: *Escherichia coli*; Flagella; GadE; Motility; Acid response; *fliC*

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Core tip: *Escherichia coli* (*E. coli*) is the number one cause of urinary tract infections in women. The infections are the result of the *E. coli* cells ascending the urinary tract via flagella presented on the outside of the cells. In this study, we have shown that *E. coli* grown in a low pH/high-osmolarity environment display transcriptional repression of the *fliC* flagellin subunit gene. Furthermore, we demonstrate that GadE may regulate *fliC* transcription and subsequent motility of the *E. coli* cells.

Citation: Schwan WR, Flohr NL, Multerer AR, Starkey JC. GadE regulates *fliC* gene transcription and motility in *Escherichia coli*. *World J Clin Infect Dis* 2020; 10(1): 14-23

URL: <https://www.wjgnet.com/2220-3176/full/v10/i1/14.htm>

DOI: <https://dx.doi.org/10.5495/wjcid.v10.i1.14>

INTRODUCTION

In the United States, approximately 10.5 million women suffer from a urinary tract infection each year. Around 80% of urinary tract infection are caused by uropathogenic *Escherichia coli* (UPEC), resulting in over 100000 hospitalizations and an approximate cost of \$ 3.5 billion per year^[1-3]. UPEC sometimes ascend all of the way to the kidneys, causing life-threatening pyelonephritis in some of the women^[2,3]. The ability of *Escherichia coli* (*E. coli*) to move up the human urinary tract is due to the presence of flagella expressed by the bacteria^[4-7].

Bacterial flagella allow the directional movement of *E. coli* based upon a chemotactic response^[8,9]. Several genes are involved in the expression of flagella, although *fliC* encodes the flagellin subunits that comprise the bulk of a flagellum structure^[10]. Several studies have shown the importance of flagella in UPEC pathogenesis^[4-7,11]. For instance, several studies have examined the prevalence of the *fliC* gene in UPEC strains. One study showed the prevalence of the *fliC* gene in UPEC strains varied from 84% (community-acquired) to 95% (nosocomial-acquired)^[12], whereas another study reported that only 16% of the UPEC strains had the *fliC* gene^[13]. Part of the disparity in the frequency of *fliC* gene prevalence could be due to the respective primers used in each study. Certainly, UPEC flagella are critical for ascension out of the bladder into the kidneys of an animal host. Within a mouse or human urinary tract, UPEC are continuously bathed in urine. Typically, human and murine urine will have a slightly acidic pH and variations in osmolality^[14-16], although the osmolality within murine urine is usually higher than human urine^[15]. Hence, pH is one critical environmental factor found in the urinary tract.

Within *E. coli*, homeostasis in an acidic environment is mediated by at least five acid response (AR) systems^[17-21]. System two (AR2) is induced in stationary phase and requires a glutamate decarboxylase and a glutamate: γ-aminobutyric acid antiporter. AR2 is the predominant and best characterized of the five AR system pathways^[22-25]. The AR2 requires the antiporter GadC and two inducible glutamate decarboxylases: GadA and GadB. The antiporter is responsible for transporting glutamate into the cell while transporting the product of glutamate decarboxylation, glutamate: γ-aminobutyric acid, out of the cell^[22,24-30]. GadE, belonging to the LuxR family of regulatory proteins^[31], has been identified as the central transcriptional activator of *gadA/BC*, and provides the primary means of *gadA/BC* activation^[32,33]. Microarray studies done under acidic conditions originally identified the *yhiE* gene (renamed *gadE*), which was found to encode for this transcriptional regulator protein^[31]. GadE binds to a 20-bp sequence (GAD box: 5'-TTAGGATTTTGTATTATAA-3') located -63 bp from the transcriptional start site of both *gadA* and the *gadBC* operon and is necessary for expression of these genes under all conditions^[28,34,35].

In this study, we have studied the role GadE may play in *E. coli* flagella expression. Through the use of a *gadE* mutant, a *fliC-lacZ* reporter system, and a motility assay; we demonstrate that GadE regulates transcription of *fliC* in *E. coli*, which in turn affects bacterial motility.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media

All of the bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strain NU149 is a clinical isolate obtained from a patient with cystitis^[36]. The *E. coli* strain DH5 α was used to construct the *fliC-lacZ* reporter system. *E. coli* strains MC4100 (supplied by Linda Kenney) and EK227 (supplied by John Foster) were subsequently tested under various pH and osmotic conditions with the *fliC-lacZ* reporter system. The Δ *gadE* strain EF1007 and Δ *gadE*/pPCRScrip Amp *gadE* strain EF1083 were also supplied by John Foster. Multicopy plasmid pUJ9^[37] and single copy plasmid pPP2-6^[38] were used for cloning. The pUJ9 plasmid contains a promoterless *lacZ* gene and an ampicillin antibiotic resistance gene. Plasmid pPP2-6 is a single copy plasmid with a multiple cloning site that possesses a chloramphenicol resistance gene^[38]. The pPCRScrip Amp *gadE* plasmid had the *gadE* gene cloned into the multicopy plasmid pPCRScrip Amp^[33]. Luria Agar (LA) supplemented with 12.5 μ g/mL chloramphenicol was used to grow the recombinant *E. coli* cells containing the reporter system. Luria Bertani (LB) broth containing 1% glycerol at pH's ranging from 5.5 to 8.0 was used to test pH ranges, and LB broth (pH 5.5 and pH 7.0, 1% glycerol, 0.1 mol/L Na₃PO₄ buffering) coupled with osmotic variation of 0 to 400 mmol/L NaCl was used to gauge pH plus osmotic changes^[38]. Under these growth conditions, the recombinant *E. coli* strains were assayed for β -galactosidase activity.

Construction of the *fliC-lacZ* fusion

Oligonucleotide primers FliC1 (5'-GAGAGAATTTCGATGAAATACTTGCCATGC-3') and FliC2 (5'-AGAAGGATCCAGACGCTGGATAGAACTC-3') specific for a 397-bp segment of the *E. coli* strain NU149 *fliC* promoter were amplified with the *Bam*HI and *Eco*RI restriction endonuclease sites flanking the DNA promoter sequence. Polymerase chain reaction (PCR) amplification using these primers was set up as follows: An initial denaturation of five minutes, then 35 cycles 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, finishing with a 7 min elongation at 72 °C after the 35th cycle. Chromosomal DNA from *E. coli* strain NU149 extracted with a PurElute Bacterial Genomic kit (Edge Biosystems, Mountain View, CA, United States) served as the template in the PCR. The 397-bp product was visualized on a 0.8% agarose gel containing ethidium bromide with a 1 kb ladder (New England Biolabs, Ipswich, MA, United States) served as the molecular weight standard.

The PCR amplified 397-bp *fliC* promoter DNA fragment was passed through a Microcon 30 filter (MilliporeSigma, Burlington, VT, United States) to concentrate the DNA. Subsequently, the DNA was digested with the restriction endonucleases *Eco*RI and *Bam*HI (New England Biolabs). The digested DNA fragment was ligated to *Eco*RI/*Bam*HI digested pUJ9 plasmid DNA and transformed into competent DH5 α cells. The resulting transformants were selected on LA containing 100 μ g/mL ampicillin and X-Gal (Promega, Madison, WI, United States). Blue colonies were screened for β -galactosidase^[39] and the plasmid DNA was extracted with a QIAprep kit (Qiagen, Valencia, CA, United States) to verify the appropriate size. One recombinant plasmid, pNK1-1, was carried further in the process. This plasmid DNA was digested with the restriction endonuclease *Not*I (New England Biolabs) and ligated to *Not*I cut pPP2-6 DNA. Following ligation, the DNA was transformed into DH5 α and clones were selected on LA containing 12.5 μ g/mL chloramphenicol and X-Gal. One clone, pNK2-29, was selected for *in vitro* analysis.

Galactosidase assays

Galactosidase assays were performed on DH5 α /pNK2-29 and MC4100/pPP2-6 cells grown in LB media at various pH and in the presence and absence of NaCl at pH 5.5 and 7.0^[39]. Bacteria were grown mid-logarithmically and β -galactosidase activity on the sodium dodecyl sulfate and CHCl₃ permeabilized cells. The mean values + standard deviation was calculated from at least three separate experiments for each bacterial strain.

Creation of a Δ *gadE* mutation in uropathogenic *E. coli* strain NU149

To create a deletion mutation of the *gadE* gene, the red recombinase system described by Datsenko and Wanner^[40] was used. Briefly, the primer pair GadE1 (5'-GATGACATATTCGAAACGATAACGGCTAAGGAGCAAGTTTGTGTAGGCTGGAGCTGCTTCG-3') and GadE2 (5'-TCGTCATGCCAGCCATGAATTTCA-GTTGCTTATGTCCTGACATATGAATATCCTCCTTAG-3') was used to create a PCR product, using pKD4 plasmid DNA as a template. The PCR conditions that were used were an initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C, 1 min; 57 °C, 1 min; and 72 °C, 2 min. The resulting PCR product was concentrated and separated on a 0.8% agarose gel, cut out, and the DNA extracted from the agarose gel.

Table 1 Bacterial strains and plasmids used in the study

Strain/plasmid	Description	Source
Strain		
DH5a MCR	Transformation efficient strain	Gibco/BRL
MC4100	<i>E. coli</i> K-12 strain	Linda Kenney
EK227	<i>E. coli</i> K-12 strain	[53]
EF1007	<i>gadE</i> ::Km in EK227	[54]
EF1083	<i>gadE</i> ::Km/pPCRScrip Amp <i>gadE</i>	[33]
NU149	Clinical isolate	[36]
NU149 <i>gadE</i>	Δ <i>gadE</i> mutation in NU149	This study
NU149 LacZ1	Δ <i>lacZ</i> mutation in NU149	This study
Plasmid		
pUJ9	Promoterless <i>lacZ</i> gene, Ap ^R	[37]
pPP2-6	Single copy plasmid, Cm ^R	[38]
pKD4	Flp recombinase sites, Km ^R	[40]
pKD46	Red recombinase, Ap ^R	[40]
pCP20	Flp recombinase, Ap ^R	[40]
pNK2-29	<i>fliC</i> :: <i>lacZ</i> on pPP2-6, Ap ^R	This study
pPCRScrip Amp <i>gadE</i>	<i>gadE</i> on pPCRScrip Amp	[33]

With this purified PCR product, an electroporation was performed on strain NU149/pKD46 cells as described previously^[40], selecting for transformants on LA with 40 µg/mL kanamycin. One transformant, NU149 *gadE*, was chosen for further analysis. To remove the kanamycin resistance gene, plasmid pCP20 was introduced into NU149 *gadE* by electroporation. The resulting strain was processed as noted previously^[6]. To confirm the *gadE* deletion, a PCR-based assay was used with the GadE5 (5'-ACAGGGCTTTTGGCAGTTGAA-3') and GadE6 (5'-AAATATTAGCGTCGACGTGA-3') primers. The PCR conditions that were used were an initial denaturation at 95 °C for 5 min followed by 30 cycles of 95 °C, 1 min; 57 °C, 1 min; and 72 °C, 2 min. This Δ *gadE* mutation was complemented by electroporating the pPCRScrip Amp *gadE* plasmid into NU149 *gadE* and selecting for transformants on LA with 100 µg/mL ampicillin. The wild-type NU149 strain was used a positive control and *Staphylococcus aureus* genomic DNA was used as a negative control.

Construction of Δ *lacZ* mutation in uropathogenic *E. coli* strain NU149

To construct the Δ *lacZ* mutation in UPEC strain NU149, the procedure described above was used. The LacZ1 (5'-CCTTACGCGAAATACGGGCAGACATGGCCTGCCCCGGTTAT

TACATATGAATATCCTCCTTAG-3') and LacZ2 (5'-TGGAATTGTGAGCGG-ATAACAA

TTTCACACAGGAAACAGCTTGTGTAGGCTGGAGCTGCTTCG-3') primer pair were used to create the PCR product using the amplification conditions noted above. To confirm the Δ *lacZ* mutation, the LacZ3 (5'-ATGAAACGCCGAGTTAACGC-3') and LacZ4 (5'-AGCTGGCGTAATAGCGAAGA-3') primers were used in the PCR amplification conditions described above. Plasmid pNK2-29 was electroporated into strain NU149 and colonies were selected on MacConkey containing 12.5 mg/mL chloramphenicol.

Soft agar assay for motility

A soft agar motility test was performed as previously described^[41] for the wild-type *vs gadE* mutant and complemented mutant analysis. Each strain was inoculated into the center of the agar plate and the amount of bacterial spread measured after 24 h post-inoculation. The motility assays were repeated two more times on separate days.

Statistical analyses

A two-tailed Student's *t*-test was used to calculate statistical variation with a *P* < 0.05 considered significant.

RESULTS

Examination of the *fliC::lacZ* fusion at different pHs

To assess whether pH affected the transcription of our *fliC-lacZ* fusion plasmid, the pH of buffered LB medium was adjusted to 5.5 to 8.0 by using 0.1 M Na₃PO₄ buffering and glycerol to maintain the pH^[38]. The resulting media were inoculated with MC4100/pNK2-29 and the β -galactosidase activities of mid-logarithmic-phase cells were determined. The optimal pH for *fliC* expression was found to be at pH 7.0 (1111 Miller units; Table 2). As the pH shifted to the acidic range, *fliC* transcription declined until there was a significant 3.9-fold difference observed comparing *fliC* transcription at pH 7.0 compared to pH 5.5 (288 Miller units, $P < 0.01$). When the pH of the buffered LB was raised into the alkaline range, there was a slight decline in *fliC* transcription that was 1.5-fold lower at pH 8.0 (738 Miller units, $P < 0.05$) *vs* growth in pH 7.0 medium. These results indicate that pH alone affects *fliC* transcription.

Effects of pH and osmotic conditions together on *fliC::lacZ* transcription

In an environment such as the human or murine urinary tract, fluctuations in both pH and osmolarity can occur^[14-16]. To determine if the combination of acidic pH and high osmolarity affect *fliC* transcription, MC4100/pNK2-29 was grown in buffered pH with variation in both the pH (5.5 and 7.0) and the osmolarity (0 to 400 mmol/L NaCl). When MC4100/pNK2-29 was grown in pH 7.0/low-osmolarity (0 mmol/L NaCl) LB, *fliC* transcription was the highest (1,132 Miller units, Table 3). An increase in the osmolarity to 400 mmol/L NaCl in the pH 7.0 LB caused *fliC* transcription to significantly fall by 2.5-fold (454 Miller units, $P < 0.01$) compared to growth in the pH 7.0 low-osmolarity LB. *E. coli* with the pNK2-29 plasmid grown in pH 5.5/low-osmolarity conditions displayed *fliC* transcription of 308 Miller units (Table 3); however, *fliC* transcription dropped almost 5-fold to 62 Miller Units ($P < 0.01$) as the osmolarity increased to 400 mmol/L NaCl. A comparison of *fliC* transcription in *E. coli* grown in pH 7.0/low-osmolarity LB to the *E. coli* population grown in pH 5.5/high-osmolarity LB showed a highly significant 18.2-fold change ($P < 0.001$). Thus, a growth environment possessing both an acidic pH and high osmolarity substantially repressed *fliC* transcription in the *E. coli* K-12 strain.

To determine if the same *fliC* transcriptional changes occurred in a UPEC strain, a Δ *lacZ* mutation was created in UPEC strain NU149. The pNK2-29 plasmid containing the *fliC-lacZ* fusion was moved into *E. coli* strain NU149 LacZ1 and the same environmental conditions tested for the *E. coli* K-12 strain were used. Growth of NU149 LacZ1/pNK2-29 in pH 7.0 with no added NaCl displayed the highest *fliC* transcription (1353 Miller Units, Table 3), whereas *fliC* transcription significantly fell 3.06-fold when the strain was grown in pH 5.5 LB (442 Miller Units, $P < 0.01$). An increase in the osmolarity to 400 mM NaCl in pH 7.0 LB caused *fliC* transcription to fall 2.77-fold (489 Miller Units, $P < 0.01$). Moreover, the growth of NU149 LacZ1/pNK2-29 in pH 5.5 LB with 400 mM added NaCl showed the lowest level of *fliC* transcription (147 Miller Units) that was 9.2-fold lower than when grown in pH 7.0 no added salt medium ($P < 0.01$). Overall, the *fliC* transcription results in the UPEC strain mirrored the *E. coli* K-12 strain's results.

Transcription of *fliC* was affected by the *gadE* mutation in *E. coli* grown in acidic pH media

As shown above, acidic pH growth conditions led to lower *fliC* transcription compared to transcription in neutral pH growth conditions. Previous work has shown that the glutamate decarboxylase system is critical for acid resistance in *E. coli* and GadE is an important regulator of this AR system^[31-33]. We then asked whether GadE might also regulate *fliC* transcription under acidic growth conditions. We examined an *E. coli* K-12 wild-type strain, a *gadE* mutant strain as well as a complemented *gadE* mutant strain all of which contained the *fliC-lacZ* pNK2-29 plasmid. The strains were grown in buffered LB set at pH 5.5 or 7.0 with (400 mmol/L) or without (0 mmol/L) added NaCl and monitored for galactosidase activity. Derepression of *fliC* transcription occurred in the *gadE* mutant grown in acidic pH LB (Table 4). After growth in pH 5.5/low-osmolarity medium, the *gadE* mutant strain (1742 Miller units) exhibited a 3.2-fold increase in *fliC* transcription, compared to the wild-type strain (540 Miller units, $P < 0.001$), which indicated that GadE repressed *fliC* under acidic conditions. Complementation with an intact *gadE* gene reduced the activity below the wild-type levels to 295 Miller units, below even wild-type levels, confirming the repressive effect of GadE on *fliC* expression. The repressive effect of GadE on *fliC* expression was reduced in pH 7.0/low-osmolarity medium with the *gadE* mutant strain showing only slightly higher *fliC* transcription (2196 Miller units) *vs* the *gadE*+ wild-type strain (1520 Miller units, $P < 0.01$). However, when the growth conditions were changed to a high osmolarity environment (400 mmol/L NaCl), the *gadE*

Table 2 Effect of pH on *fliC::lacZ* gene transcription in *Escherichia coli* strain MC4100/pNK2-29 grown in buffered Luria Bertani media

pH	Gal activity ¹
5.5	288 ± 81.5
6	528 ± 82.5
6.5	629 ± 114
7	1111 ± 110
7.5	932 ± 190
8	738 ± 125

¹Galactosidase activity measured as Miller units.

mutation had no significant effect on *fliC* transcription (540 Miller units). A change to a pH 5.5/ high-osmolarity environment caused a further repression of *fliC* transcription (165 Miller units, $P < 0.05$) that was significant.

A *gadE* mutation affects uropathogenic *E. coli* motility

The data above suggested that GadE may repress *fliC* transcription when *E. coli* is grown under acidic pH conditions. Since transcriptional differences do not always translate into protein level differences or functional differences, the effects of a *gadE* mutation on *E. coli* motility was next tested. First, motility was tested using the *E. coli* K-12 strain EF227 (wild-type), EK1007 (*gadE* mutation), and EF1083 (*gadE* mutation complemented with the pPCRScrip Amp *gadE* plasmid). All strains were grown in pH 5.5 buffered LB and spotted onto motility agar plates. Wild-type *E. coli* strain EF227 displayed an 8.33 mm spread diameter, whereas strain EF1007 showed a significantly larger spread diameter of 45 mm ($P < 0.001$, Table 5). When the *gadE* mutation was complemented in strain 1083, the spread diameter dropped below the wild-type level (6.67 mm diameter).

A *gadE* mutation was also created in the uropathogenic *E. coli* clinical isolate NU149 using a λ red recombinase system. The NU149, NU149 *gadE*, and NU149 *gadE*/pPCRScrip Amp *gadE* strains were grown in pH 5.5 buffered LB and spotted onto motility agar plates. Wild-type *E. coli* strain NU149 had a 10.67 mm spread diameter, whereas strain NU149 *gadE* had a 57.34 mm spread diameter that was significantly wider ($P < 0.05$). Complementation of the *gadE* mutation brought the spread diameter back down to a wild-type level (7.00 mm). These results indicate that GadE also affects UPEC motility.

DISCUSSION

The production of flagella in UPEC is vital for their pathogenesis in a human host, enabling the bacteria to ascend the urinary tract^[4-7,11]. A transcriptome study of a UPEC strain in the murine urinary tract over time demonstrated that several genes that are involved in flagella biosynthesis and chemotaxis, including the *fliC* structural gene, had their transcription down-regulated in this environment^[42]. Within the urinary tract, the *E. coli* encounter an environment that typically has a slightly acidic pH and osmotic changes that increase as the bacteria move into the kidneys of the host^[14-16]. *E. coli* is able to survive in acidic pH environments that include the human and murine urinary tracts because of AR systems that include the glutamate decarboxylase system^[15-18]. GadE is an important protein that regulates this AR system^[31-33]. Since GadE is important for regulating genes in one AR system, could the GadE regulator of the glutamate decarboxylase AR system also be involved in the down-regulation of *fliC* in uropathogenic *E. coli* growing in the murine urinary tract?

To answer the question above, we designed a *fliC-lacZ* reporter system on a single copy number plasmid to measure *fliC* transcription within *E. coli* growing in various environments that might be encountered in the urinary tract. Our results showed *fliC* transcription fell in both *E. coli* strains grown in a pH 5.5 environment compared to a neutral pH environment, suggesting one or more proteins produced by *E. coli* growing in an acidic pH environment represses *fliC* transcription. A previous study revealed a substantial drop in motility by *E. coli* grown in an acidic environment *vs* a neutral pH environment^[43] that correlates with our experimental observations in this study. Moreover, *E. coli* growth in a high salt concentration medium also caused repression of *fliC* transcription. Li *et al*^[44] observed that *E. coli* grown in a high-

Table 3 Effect of osmolarity on *fliC::lacZ* gene transcription in *Escherichia coli* grown in buffered pH 5.5 and 7.0 Luria Bertani media with different osmolarities

<i>E. coli</i> strain	NaCl (mmol/L)	Gal activity ¹	
		pH 5.5	pH 7.0
MC4100/pNK2-29	0	308 ± 104 ²	1132 ± 130
MC4100/pNK2-29	100	338 ± 128	806 ± 41
MC4100/pNK2-29	200	251 ± 68.5	689 ± 173
MC4100/pNK2-29	400	62 ± 22.0	454 ± 71
NU149 LacZ1/pNK2-29	0	442 ± 72	1353 ± 98
NU149 LacZ1/pNK2-29	100	418 ± 61	976 ± 52
NU149 LacZ1/pNK2-29	200	293 ± 43	811 ± 75
NU149 LacZ1/pNK2-29	400	147 ± 39	489 ± 61

¹Galactosidase activity measured as Miller units.²Data represents the mean ± standard deviation from three separate runs.

osmolarity medium were less motile compared to *E. coli* grown in a low-osmolarity medium.

A combination of pH changes and osmolarity changes was also examined using our *fliC-lacZ* system. In a low pH/high-osmolarity medium, the growing *E. coli* exhibited an additive level of repression of *fliC* transcription that is in line with the previous transcriptome study^[42].

Two environmental variables are at play in a low pH/high-osmolarity environment. To adapt to acidic pH conditions, *E. coli* rely on AR systems and their corresponding regulators, such as GadE. On the other hand, the OmpR-EnvZ two-component system is the main osmotic stress regulatory system in *E. coli*^[45]. OmpR has been shown to regulate flagella expression^[46,47] and is likely partially responsible for repressing *fliC* transcription in the high-osmolarity environment that we tested. Furthermore, OmpR-regulated genes are tied to the acid response in *E. coli* and *Salmonella enterica*^[48,49].

Since GadE is a central player in AR system regulation, we examined *fliC* transcription and motility in *gadE* mutant strains *vs* the wild-type strains. By deleting the *gadE* gene, *E. coli* *fliC* transcription was derepressed, particularly in *E. coli* growing in an acidic pH environment. Complementation of the *gadE* mutation with the *gadE* gene on a multicopy plasmid caused additional suppression of *fliC* transcription that was below wild-type levels. Furthermore, a Δ *gadE* mutation in K-12 and UPEC strains led to significantly greater motility compared to the wild-type strain. Together, these data suggest that GadE represses *fliC* transcription either by directly binding to the *fliC* promoter to repress transcription or acting in an indirect manner by influencing expression of FlhD that in turn regulates *fliC*^[50,51]. However, GadE does not appear to affect osmotic control of *fliC* transcription.

What would be the advantage of a loss of flagella expression in *E. coli* growing in the human kidney? Flagella protruding from the surface of *E. coli* cells represent a target of the host's immune system. Flagellated *E. coli* cells are more likely to be phagocytized than no-flagellated cells^[52]. *E. coli* that have reached the kidneys would be in a low pH/high-osmolarity environment where the flagella are no longer needed and may in fact be a detriment to their survival. Through the regulatory effects of the GadE and OmpR proteins, *fliC* transcription may be shut down, causing the bacterial cells to lose their flagella and be able to hide behind their anti-phagocytic capsules.

Table 4 Assessing a *gadE* and mutation and complementation on *fliC::lacZ* gene transcription in *Escherichia coli* grown in buffered pH 5.5 and 7.0 Luria Bertani media with different osmolarities

<i>E. coli</i> strain	Gal activity ¹			
	pH 5.5	pH 5.5 ²	pH 7.0	pH 7.0
EK227/pNK2-29	540 ± 51 ³	165 ± 59	1520 ± 144	540 ± 66
EF1007/pNK2-29 ⁴	1742 ± 109	470 ± 106	2196 ± 173	681 ± 135
EF1083/pNK2-29	295 ± 93	131 ± 20	794 ± 145	404 ± 41

¹Galactosidase activity measured as Miller units.²400 mmol/L added NaCl.³Data represents the mean ± standard deviation from three separate runs.⁴EF1007 is *gadE* and EF1083 is *gadE*/pGadE+.**Table 5 Motility of *Escherichia coli* strains NU149 and EK227, their *gadE* mutants, and complemented *gadE* mutants grown in pH 5.5 Luria Bertani**

Strain	Motility (mm) ¹
NU149	10.67 ± 1.25 ²
NU149 <i>gadE</i>	57.34 ± 10.21
NU149 <i>gadE</i> /pPCRScrip <i>gadE</i>	7.00 ± 0.82
EK227	8.33 ± 1.52
EF1007 (<i>gadE</i>)	45.00 ± 2.00
EF1083 (<i>gadE</i> /pPCRScrip <i>gadE</i>)	6.67 ± 1.53

¹Spread diameter after 24 h on a motility plate measured in mm.²Data represents the mean ± standard deviation from three separate runs.

ARTICLE HIGHLIGHTS

Research background

Uropathogenic *Escherichia coli* (UPEC) is the number one cause of urinary tract infection in women. Motility driven by the action of flagella is critical for UPEC pathogenesis. How *Escherichia coli* (*E. coli*) adapts to a low pH/high osmolarity environment is essential for the species survival. Acid tolerance systems, such as the System two system, are important for UPEC survival in a low pH environment.

Research motivation

Our key problem to be solved was whether GadE, a part of the acid response two system, regulates transcription of the *fliC* gene, and in turn, UPEC motility.

Research objectives

Determine whether GadE regulated *fliC* transcription and subsequent motility of the *E. coli*.

Research methods

We created a *fliC-lacZ* reporter system on a single-copy number plasmid and measured b-galactosidase levels in both a K-12 and UPEC clinical isolate. Furthermore, motility was assessed in both *E. coli* strains by inoculating wild-type, *gadE* mutant, and complemented *gadE* mutant strains onto motility agar.

Research results

Transcription of *fliC* was significantly lower in *E. coli* grown in pH 5.5 Luria Bertani compared to pH 7.0 Luria Bertani. A mutation in the *gadE* gene led to higher *fliC* expression in that strain *vs* wild-type bacteria. Motility was significantly higher in the *gadE* mutant strain compared to the wild-type strain.

Research conclusions

We confirmed that *fliC* transcription was down-regulated in *E. coli* grown in a low pH/high osmolarity environment compared to a neutral pH/low osmolarity environment. GadE appears to either directly or indirectly regulate *fliC* transcription in *E. coli*.

Research perspectives

Future work could be done to affirm the GadE regulation of flagella expression in *E. coli*.

ACKNOWLEDGEMENTS

The authors wish to thank John Foster for a critical reading of the manuscript. We wish to thank Linda Kenney (MC4100) and John Foster (EK227, EF1007, EF1083) for strains used in this study.

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