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Comparative Analysis of SARS-Cov-2 Detection Using Viral Swab in Viral Transport Medium Against Cotton Swab in 0.9% Normal Saline

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) has become a major health problem causing severe acute respiratory illness in humans. With the high case count and mortality rate, a broad testing method is required to detect the virus in infected people, as well as less common clinical manifestations of the disease. Consequently, the high demand for testing has resulted in a depletion of commercially available consumables, including the recommended swabs and viral transport media (VTM) required for oropharyngeal sampling. To address this issue, we evaluated the utility of a commonly found cotton swab in 0.9% normal saline against the viral swab in viral transport medium (VTM) for the molecular detection of SARS-CoV-2. The study was a cross-sectional analytical study that recruited 322 suspected COVID-19 patients from Kwadaso Seventh Day Adventist and Suntreso Government Hospitals, Kumasi, Ghana, between April and September 2021. Consecutive oropharyngeal swab samples were obtained with viral swabs and cotton swabs in parallel and inserted into the viral transport medium and 0.9% normal saline, respectively. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed on the samples concurrently for detection of SARS-CoV-2 genes (N and ORF1ab genes). Comparison of the cotton swab in 0.9% saline samples to the viral swab in VTM samples, yielded the following results: the cotton swab samples were 61.9% (51.7-71.2) sensitive, 96.9% (93.8- 98.5) specific, and with positive and negative predictive values of 89.0% and 86.4% respectively. The median viral load was significantly higher in samples taken with a viral swab in VTM (276, IQR: 51.49- 9837.87) compared to samples taken with a cotton swab in 0.9% saline (252.86, IQR: 29.62-4235.93), p = 0.0059. Our study suggests that cotton swabs in 0.9% saline have low sensitivity and viral yield and hence not appropriate for collection of respiratory samples for SARS-CoV-2 detection.

Keywords: SARS-CoV-2; COVID-19; Oropharyngeal sampling; Cotton swab; 0.9% Normal salin

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INTRODUCTION

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), was initially reported in Wuhan, China, during the late 2019 [1]. The 30 kilobase enclosed, single-stranded, positive-sense RNA virus is widely dispersed in humans and mammals [2], and rapidly progressed to a global pandemic in March 2020. SARS-CoV-2 has a median reproductive number (R0) of 2.5, with some variants reaching as high as 6.09 [3], infecting nearly 619 million people and causing as much as 6 million associated deaths globally as of October 2022 [4]. The highest case count is reported in Europe (256,938,830), with the Americas recording the highest associated deaths (2,843,705) within the same period [4].

In comparison to other continents, Africa has recorded relatively lower case and death counts. The continent has, as of October 12, 2022, recorded 9,338,726 cases with 174,568 associated deaths. Ghana, located in the West Africa, recorded the earliest two (2) cases on 12th March 2020 [5], but currently has 170,177 reported cases as well as 1,460 associated deaths [6].

For clinical management and outbreak control, collecting proper respiratory specimen, preservation, transportation and testing samples of patients that meet the suspect case criteria for SARS-CoV-2 is considered precedence. SARS-CoV-2 detection is mainly done in the laboratory by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) in lower or upper respiratory samples. Notably, nasopharyngeal (NP) or oropharyngeal (OP) swabs taken in viral transport medium (VTM) have been the most widely used samples for COVID-19 diagnosis [7]. For OP/NP swab collection, it is recommended to use synthetic fiber swabs with plastic shafts as they do not interfere with virus inactivation or polymerase chain reaction (PCR) compared to calcium alginate or wooden shaft swabs and conveyed to the lab in VTM [7]. VTM is used as a standard medium for maintaining the integrity of samples meant for molecular-based assays.

Globally, increased testing has put a strain on specimen collection and transportation media supplies, particularly, personal protection equipment (PPE), viral swabs and VTM, with low-income and resource settings bearing the brunt of the burden. Due to the heightened demand, cotton swabs, phosphate buffered saline (PBS), and 0.9% saline have been used as an alternative toviral swabs for sample collection in resource-burden countries like Ghana, which increases the risk of reduced viral detection and misdiagnosis [8-10]. Studies that assessed the stability of other viral pathogens in different swabs and transport media [11-14] suggested traditionally flocked nylon, rayon, spun polyester, and cotton swabs as possessing equivalent viral preservation stability [15]. While this alternative approach appears feasible, there is limited data on clinical utility of cotton swabs in physiological saline for SARS-CoV-2 detection as compared with other pathogens. Although 0.9% saline is known to prevent the release of intracellular RNase [16], it is not clear whether its isotonic nature preserves RNA or other nucleic acid material. This study sought to compare the diagnostic performance of cotton swabs in 0.9% normal saline to the widely recommended viral swab in VTM for SARS-CoV-2 detection among suspected COVID-19 patients.

METHODOLOGY

Study Design and Participants

This was a hospital-based cross-sectional study conducted between April and September 2021. Eligible participants were of every age group suspected of SARS-CoV-2 infection or presented to the hospitals with any symptoms of the disease. Participants were conveniently selected as they were presented to the two (2) hospitals.

Study Setting

The study was performed at the Suntreso Government Hospital (SGH) and the Kwadaso Seventh Day Adventist Hospital (KWA), all stationed in Kumasi, Ashanti region, Ghana. Kumasi is considered the second largest and crowded city within Ghana after Accra, with a populace around 3,353,850 individuals as of 2021 accommodating over 200 health facilities [17]. Both hospitals are among the leading providers of high-quality healthcare in Kumasi, with SGH and KWA serving as a district and referral hospitals within the North Suntreso and Kwadaso districts. Their services focus primarily on their communities but extend to other neighbouring communities. SGH is located in Bantama, North Suntreso of Kumasi whereas KWA can be found in Kwadaso. Both recruitment sites were relatively among the readily and easily accessed hospitals in the metropolis during the pandemic's pinnacle.

All laboratory procedures were done at the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) of KNUST, situated in Kumasi, Ghana. The center, which is a well-known research facility with various clinical laboratories, was a collective initiative between agencies in Ghana and Germany in 1997. The test center was one of the many COVID-19 diagnostic laboratories in Ghana with around 1000 tests per diem [18].

Oropharyngeal Swab Sampling

All suspected COVID-19 cases were taken at a designated area at the hospital following safety protocols. For each participant, two (2) consecutive oropharyngeal swabs were taken by swabbing the back of the throat near the tonsils,

the posterior oropharynx, and subsequent swollen areas thoroughly recurrently by qualified medical personnel. The first swab was obtained using an aseptic viral swab (Bioteke Corporation, ST9001-1 model) and placed in a tube containing sterile VTM (Bioteke Corporation, ST9001-1 model). The swab shaft was broken against the side of the tube gently to avoid splashing the contents and the top of the swab discarded. The second swab was taken using a sterile cotton swab (Sarstedt, Barcelona, Spain) and placed in a 2-ml cryogenic tube (Qingdao Haver Biomedical Co. Ltd., China) containing 0.9% normal saline, and the shaft broken and placed in the saline. The samples were appropriately labelled, placed in a sterile biosafety bag and transported in a cold box (2-8°C) in a triple package within an hour of collection to KCCR for laboratory analysis.

Laboratory Analysis

The viral ribonucleic acids were extracted from the samples using the QIAamp Viral RNA Mini Kits (Qiagen, Hilden, Germany) and the spin column method, per the manufacturer's protocol. Five microliters (5µL) of the extracted RNAs were amplified through a one-step reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) method and ORF1ab and N genes of SARS-CoV- 2 genome quantitatively detected using the Applied Biosystems 7500 Fast System (Thermo Fisher Scientific, Singapore) thermocycler using the cycling conditions: reverse transcription at 50°C for 15 mins (1 cycle), initial denaturation at 95°C for 15 mins (1cycle) and 45 cycles each of denaturation and annealing at 94°C and 55°C for 15 seconds and 45 seconds. For each test run, a positive and negative control were added for validation. Samples with Ct value ≤ 40 were regarded positive for the 2019-nCOV. Viral loads were deduced from a standard curve generated from plotting known viral concentrations against the Ct values of a target.

Data Collection and Analysis

Patient information was taken using the Ghana Health Service and W.H.O approved COVID-19 Case Investigation Form designed in sections. Data were first entered into Microsoft Excel for Mac (version 16.63.1) and exported to IBM SPSS Statistics 25 to determine associations among variables determined using the chi-square test. Contingency tables were used to determine the sensitivity, specificity, and predictive values. A correlation in addition was represented diagrammatically using simple linear regression. Test viral loads were illustrated using scatterplots and Kendall coefficient of concordance, W for positive cases. A p-value ≤ 0.05 was regarded statistically significant for all analysis.

RESULTS

Sociodemographic and Clinical Characteristics of Study Participants

Table 1 depicts sociodemographic and clinical factors associated with COVID-19 status. Of the 322 participants recruited into the study, 27.0% were within the age range of 30-39 years and 21.4% were 20-29 years. More than half (56.8%) of the participants were females and two-thirds (66.5%) were asymptomatic for COVID-19. The majority (89.3%) had mild to moderate symptoms with predominant clinical symptoms being cough (41.9%), headaches (34.5%), fatigue or general weakness (31.7%) and fever (25.2%). This study found age group ($p = 0.024$), clinical symptoms ($p < 0.0001$), such as cough ($p = 0.018$) to be significantly linked to COVID-19 status. Nonetheless, no significant association between gender ($p = 0.859$), clinical symptoms such as headache ($p = 0.553$), fatigue ($p = 0.449$), loss of smell and taste ($p = 0.492$), fever ($p = 0.542$) and COVID-19 status were observed.

Distribution and Comparison Of COVID-19 Status Between Viral Swab in VTM and Cotton Swab in 0.9% Saline

Distribution of COVID-19 status between viral swabs in VTM and cotton swabs in 0.9% saline. Using viral swab in VTM produced higher positivity (28.6%) as compared with cotton swab in 0.9% saline (19.9%).

Diagnostic Performance of Cotton Swab in 0.9% Saline Compared to Viral Swab in VTM Samples

When the performance of the cotton swab in 0.9% saline samples was compared to the viral swab in VTM samples, the cotton swab samples were 61.9% sensitive, 96.9% specific, and with positive and negative predictive values of 89.0% and 86.4% (Table 2).

Table 1: Sociodemographic and clinical factors associated with COVID-19 status

Variable	Total	COVID-19 Negative	COVID-19 Positive	*p*-value
Age Group (years)				**0.024**
< 20	37 (11.5)	33 (14.3)	4 (4.3)	
20-29	69 (21.4)	52 (22.6)	17 (18.5)	
30-39	87 (27.0)	61 (26.5)	26 (28.3)	
40-49	53 (16.5)	38 (16.5)	15 (16.3)	
50-59	30 (9.3)	21 (9.1)	9 (9.8)	
60 and above	46 (14.3)	25 (10.9)	21 (22.8)	
Gender				0.859
Female	183 (56.8)	130 (56.5)	53 (57.6)	
Male	139 (43.2)	100 (43.5)	39 (42.4)	
Clinical symptoms				**< 0.0001**
Asymptomatic	108 (33.5)	95 (41.3)	13 (14.1)	
Symptomatic	214 (66.5)	135 (58.7)	79 (85.9)	
Specific Clinical Symptoms				
Fever	81 (25.2)	60 (26.1)	21 (22.8)	0.542
Fatigue/general weakness	102 (31.7)	70 (30.4)	32 (34.8)	0.449
Cough	135 (41.9)	87 (37.8)	48 (52.2)	**0.018**
Runny nose	64 (19.9)	48 (20.9)	16 (17.4)	0.480
Headache	111 (34.5)	77 (33.5)	34 (37.0)	0.553
Loss of smell and taste	69 (21.4)	47 (20.4)	22 (23.9)	0.492
Chest pain	55 (17.1)	44 (19.1)	11 (12.1)	0.122
Joint pains or Arthritis	13 (4.0)	11 (4.8)	2 (2.2)	0.283
Abdominal pain	3 (0.9)	2 (0.9)	1 (1.1)	0.854
Nausea/Vomiting	24 (7.5)	19 (8.3)	5 (5.4)	0.383
Sore throat/Pharyngitis	42 (13.0)	29 (12.6)	13 (14.1)	0.714
Chills/Sweats	23 (7.1)	15 (6.5)	8 (8.7)	0.494
Diarrhea	12 (3.7)	7 (3.0)	5 (5.4)	0.306
Dyspnea	23 (23)	13 (5.7)	10 (10.9)	0.101
Bitter mouth	3 (0.9)	2 (0.9)	1 (1.1)	0.854
Myalgia/Muscle pains	10 (3.1)	5 (2.2)	5 (5.4)	0.128
Others	13 (4.0)	9 (3.9)	4 (4.3)	0.858

Data is presented as frequency with the corresponding percentage in parenthesis. p is significant at $\leq$ 0.05.

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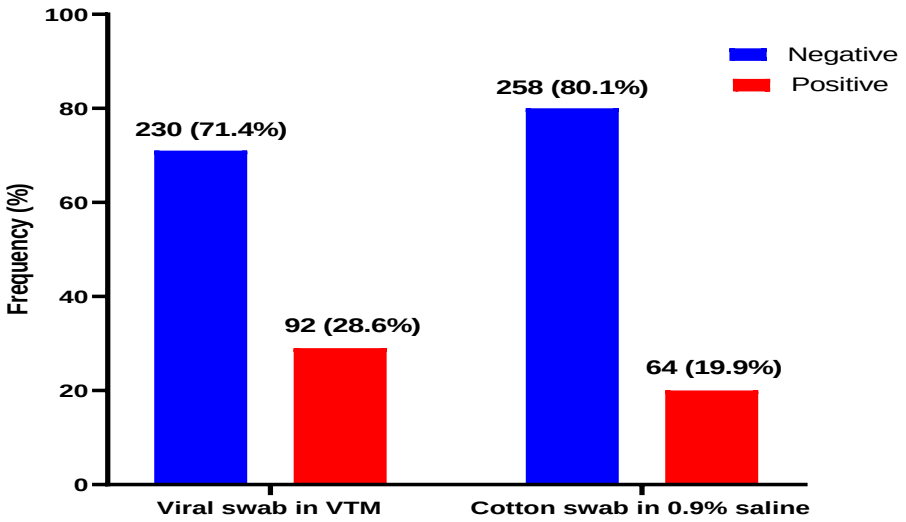

Figure 1: Distribution of COVID-19 status between viral swab in VTM and cotton swab in 0.9% saline.

Table 2: Diagnostic performance of cotton swab in 0.9% saline compared to viral swab in VTM samples

		Viral Swab in Viral Transport Media		Total	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV%	NPV%
		Pos	Neg					
Cotton Swab in 0.9% Saline	**Pos**	57(62.0)	7(3.0)	64(19.9)	61.9 (51.7-71.2)	96.9 (93.8-98.5)	89.0	86.4
	Neg	35(38.0)	223(97.0)	258(80.1)				
	Total	92(28.6)	230 (71.4)					

Pos: Positive, Neg: Negative, CI: Confidence Interval, PPV: Positive Predictive Value, NPV: Negative Predictive Value

Comparison of Viral Load Between Viral Swab in VTM and Cotton Swab in 0.9% Saline Samples

Test viral loads from a viral swab in VTM and cotton swab in 0.9% saline were illustrated using scatterplots and Kendall coefficient of concordance among participants who tested positive for COVID-19 (Figure 2). A positive correlation between viral swabs in VTM and cotton swabs in 0.9% saline samples was observed. The Kendall coefficient of concordance was 0.473, indicating that the viral load was moderately equivalent between samples taken by viral swab in VTM and those taken with a cotton swab in 0.9% saline.

The median viral load was significantly higher in samples taken with a viral swab in VTM compared to samples taken with a cotton swab in 0.9% saline [$p = 0.0059$].

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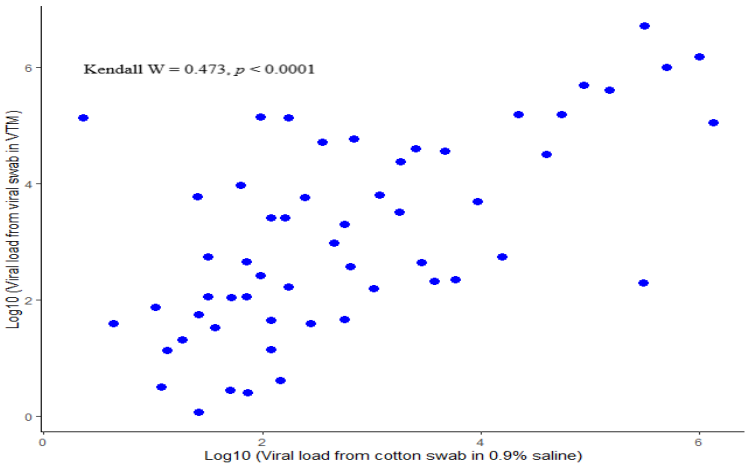

Figure 2: Comparison of viral load between viral swab in VTM and cotton swab in 0.9% saline samples; Viral loads were log-transformed to base 10. p is significant at ≤ 0.05

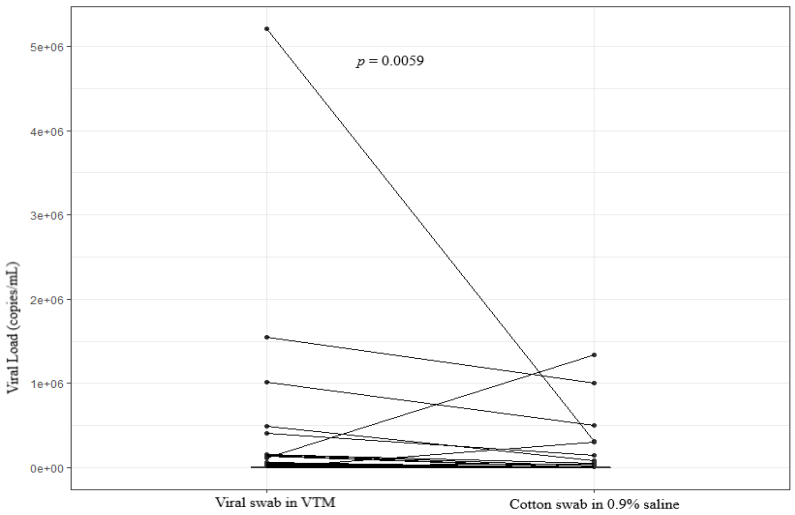

Figure 3: Comparison of SARS-CoV-2 loads between viral swab in VTM and cotton swab in 0.9% saline samples. The median viral loads ("copies/mL") were significantly higher in viral swab in VTM than in 0.9% saline (p = 0.0059).

DISCUSSION

Findings from this study showed patients in their 30s to be most positive for COVID-19. The positivity rate observed in this study for the 30-39 age range is in concordance with other works that evaluated the epidemiological profiles of participants with SARS-CoV-2 in some regions within Ghana [3, 19]. Adolescents were likely more active and frequently engaged in alfresco activities and barely observed protective measures, ensuing in a higher case surge. However, it fails to explain why participants aged 60 years and above had higher positive cases than those in their 20s and below. The low positive case observed in the participants in this age-group had been perceived in other surveys [20, 21]. One possible explanation is that the outbreak brought about strict quarantine procedures and the closure of potential hotspots for pediatric infections, such as schools and day-care centers, restricting the movement of children and increasing the spread by infected adults. In some cases, individuals within families diagnosed with SARS-CoV-2 infection were isolated outside their homes, reducing children's vulnerability to the virus [21]. Notwithstanding, this result indicates that all groups are at risk of contracting the virus. The females in this study had a higher number of

positive cases for COVID-19 in comparison to males. Although studies have indicated a higher susceptibility of males to the infection [3, 22, 23], that of females to males [24], accumulating epidemiological evidence shows no sex or gender disparities in viral susceptibility [25, 26]. The dissimilarity in observation could be due to the sampling types, areas, and methods employed.
Approximately 85.9% of positive cases in the study expressed symptoms, predominantly, fatigue/general weakness, chest pain, cough, fever, dyspnea, loss of taste and smell. This is similar to other works which reported high percentages of participants expressing symptoms [27, 28]. Although this is inconsistent with the global trend, in which more than 80% of cases are asymptomatic [29], the hospital-based nature of this study is the most likely reason there are more symptomatic patients.

In this study detecting SARS-CoV-2 RNA from oropharyngeal swab using the cotton swab in 0.9% normal saline demonstrated a sensitivity and a specificity of 61.9% and 96.9% respectively. The sensitivity observed in this study is lower compared to other studies [10]. Owing to the extremity and nature of COVID-19, a highly sensitive test that produces few false negative results is recommended. COVID-19 has a high case fatality ratio [30], so assays for identifying the virus must be highly sensitive. According to the finding of this study, collecting viral samples with cotton swabs and transporting it in 0.9% normal saline may result in misdiagnosis.

In comparison to an oropharyngeal sample taken with a viral swab and transported via VTM, we found a significantly lower SARS-CoV-2 viral load recovered when using cotton swab in 0.9% normal saline**.** This is in agreement with a study that compared cotton and flocked swabs and discovered that cotton swabs significantly yielded lower viral [9]. Garnett et al. [12] also compared the capabilities of six (6) swabs and different transport media readily available in healthcare settings. They compared Dulbecco's Modified Eagle Medium (DMEM), PBS, 0.9% normal saline, and 100% ethanol as alternatives to VTM and realized SARS-CoV-2 viral load was significantly lowest in 0.9% normal saline [12]. In another study, there was no evidence of loss of stability and sensitivity in the 0.9% normal saline used [14]. The difference in observation could be attributed to the differences in sampling, as testing was done in actual patients whereas theirs was in viral cultures.

VTMs are made up of heat inactivated Fetal Bovine Serum (FBS) which serve as proteins or amino acid source to stabilize the virions and eliminate complements. The presence of antibiotics also provides protection against antimicrobial contamination, contributing to viral RNA/DNA preservation. The isotonic solution of normal saline together with the cotton swab is one potential reason the stability and viral load of SARS-CoV-2 RNA was significantly decreased.

CONCLUSION

The results from this study indicated a significant loss of viral load, decreased test sensitivity and specificity and a decreased positivity when sampling was done with cotton swabs and transported in 0.9% normal saline for COVID-19 diagnosis. This study provides preliminary corroboration that oropharyngeal swabs collected with more generally accessible, consumer-grade, cotton-tipped swabs and preserved in a 0.9% normal saline cannot be utilized for SARS-CoV-2 detection in clinical environments as an alternative to the recommended viral swabs and VTM.

ABBREVIATIONS

COVID-19	Coronavirus Disease 2019
Ct	Cycle threshold
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
NP	Nasopharyngeal
ORF	Open Reading Frame
OP	Oropharyngeal
PBS	Phosphate Buffered Saline
RT-qPCR	Reverse Transcription quantitative Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
VTM	Viral Transport Medium

ETHICS APPROVAL

Ethical clearance for the study was obtained from the Committee for Human Research Publication and Ethics (CHRPE) of the School of Medicine and Dentistry, KNUST, Kumasi, Ghana (CHRPE/AP/076/21).

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

FUNDING

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTION

MO, AA, AAS, ROP and YMD designed the study. AA, EA, JAA, SA, CA, GA, JSK, MAB were involved in the collection of samples for testing, AAS, MM, MO and ROB were involved in the validation of results, AA, EA, GA were involved in

the data analysis. All authors were involved in the preparation and writeup of the manuscript. MM, ROP, AAS and MO reviewed the final manuscript for submission.

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