



Routes of Transmission of Newly Emerging SARS-CoV-2: A Systematic Review

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Abstract

Background: In December 2019, a novel corona virus (SARS-CoV-2) causes atypical pneumonia now known as “corona virus disease 2019” (COVID-19) emerged in Wuhan, China and spread rapidly throughout the world. **Objective:** The purpose of the present study was to identify the different plausible routes of transmission of SARS-CoV-2 into the human body. **Methodology:** We searched electronic databases (MEDLINE, EMBASE, Science Citation Index, WHO reports and news) checked documents and references. We included all the studies regarding potential routes of transmission of SARS-CoV-2. All reviewers independently screened titles and abstracts, assessed studies for inclusion, appraised quality, and extracted data. **Result:** SARS-CoV-2 has been found to have higher level of transmissibility than other two pathogenic CoVs. Sustained human to human transmission of SARS-CoV-2 has already been established though the exact route of transmission is yet to be determined. Similar to other CoVs, respiratory droplet and contact with contaminated surfaces are considered as principle route of transmission of SARS-CoV-2 whereas aerosol, feco-oral, perinatal, ocular surface and transfusion transmissions are the plausible route of spreading of this highly pathogenic CoVs. **Conclusion:** In the current review, we summarize the potential route of transmission of SARS-CoV-2 in the light of previous SARS-CoV and MERS-CoV outbreaks to solidify the scientific basis on which important decisions can be made. [*Bangladesh Journal of Infectious Diseases, April 2020;7(suppl_1):S18-S31*]

Keywords: Routes of transmission; newly emerging; SARS-CoV-2; systematic review

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Introduction

The recent emergence of a novel coronavirus of zoonotic origin in Wuhan, Hubei province, China, in December 2019, has been posing widespread fear and significant threat to global health security due to its rapid national and international spread¹. The virus was officially designated as SARS-CoronaVirus-2 (SARS-CoV-2), causing severe respiratory tract infections in humans, leading to a condition that has been entitled as “coronavirus disease 2019” (COVID-19)². As of April 2, 2020, a total of 9,40,622 cases of COVID-19 have been confirmed by nucleic acid testing of respiratory tract samples and it has caused 47,516 deaths worldwide³. WHO declared this ongoing outbreak as “Pandemic” on 11 March 2020⁴. Besides, current outbreak of SARS-CoV-2, the world has experienced the devastating effect of another two outbreaks caused by two potentially dangerous zoonotic corona viruses in the past two decades. The first coronavirus outbreak was caused by Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002-2003⁵ and second outbreak was caused by Middle East Respiratory Syndrome coronavirus (MERS-CoV) in 2012⁶.

Corona viruses were discovered in 1960s, belonging to the family Coronaviridae, within the order Nidovirales that are widely detected in mammals and birds. Coronaviridae family consisted of two subfamilies-Orthocoronavirinae and Torovirinae. There are four genera: alpha-, beta-, gamma- and delta-coronavirus, included in Orthocoronavirinae subfamily⁷. Including SARS-CoV-2, seven human coronaviruses have been identified so far. Among them 229E, OC43, NL63, and HKU1 cause common cold symptoms in immunocompetent individuals⁸ and two other SARS-CoV and MERS-CoV are zoonotic in origin and lead to severe and potentially fatal infection⁹. Before emergence of SARS-CoV in 2002, coronavirus did not get so much attention as it causes mild symptoms in most of the cases¹⁰. First epidemic of 21st century by SARS-CoV in 2002, major outbreak of MERS-CoV in 2012 and another ongoing pandemic by SARS-CoV-2 have revolutionized the epidemiological concept of coronavirus¹¹.

Like other respiratory viruses’ corona viruses transmitted from person to person by respiratory droplets. SARS-CoV and MERS-CoV also have the potential to be transmitted by contaminated fomites and droplet nuclei or aerosol¹². SARS-CoV-2 has some similarities with SARS-CoV but it is spreading faster, assuming that it is more contagious than SARS-CoV¹³. Person to person transmission of SARS-CoV-2 in hospital and family settings has been documented albeit the route of transmission is not clearly defined so far^{14,15,16}. As number of cases of ongoing pandemic of COVID-19 is going rapidly and death toll breaking the previous days records every day, it is high time for strategic preparedness to mitigate the adverse effect of COVID-19 and to contain the SARS-CoV-2 in countries where its infection rate is still within limits. The COVID-19

pandemic could have infected 90% of the world population and killed 40.6 million people if no mitigation measures are taken right now to combat. Only by taking protective measures early to prevent spreading when the case fatality rate of an individual's country is 0.2 per 100,000 people per week may cut worldwide death to 1.9 million¹⁷.

Hence, sound knowledge regarding spatio-temporal transmission patterns of SARS-CoV-2 is thought to be central in developing strategies to control them, especially when its biological information is limited and no vaccine and treatment options are available. In the current review, we summarize the potential route of transmission of SARS-CoV-2 in the light of previous SARS-CoV and MERS-CoV outbreaks.

Methodology

Types of studies included

We included any study related to different routes of transmission of SARS-CoV-2 virus in a population-based or hospital-based sample. For pragmatic reasons, we also included different ways of transmission of SARS and MERS Coronavirus in different settings.

Identification and Selection of Studies

We had searched the electronic databases, MEDLINE, EMBASE, Science Citation Index, WHO reports, news (up to 30th March, 2020). We had searched using the keywords, always in combination with “SARS-CoV-2” or “novel coronavirus 2019”, “SARS” or “MERS” and “respiratory droplet”, “aerosol transmission”, “persistence in environment”, “survival surface”, “survival in clothes and shoes”, “perinatal transmission”, “feco-oral transmission”, “ocular surface transmission”, “transfusion transmission”, “potential route of transmission” and the full text of any articles that appeared relevant was retrieved. We also searched for relevant studies by screening titles and abstracts listed in the WHO SARS-CoV-2 bibliography, by checking lists of references in relevant studies (all included studies and reviews), editorial, and personal communication.

Data Extraction and Assessment of Methodological Quality

Relevant data from each of the individual studies were extracted by one reviewer and verified by other reviewers using the predesigned data extraction form to capture the study characteristics and the outcome of interest. The study characteristics included information about the origin of the study, the period of evaluation, the population and types of laboratory tests to confirm the diseases. The outcome of interest was the risk of transmission of SARS-CoV-2, SARS-CoV and MERS-CoV infections from patients to HCWs (health care workers) and general populations. Any disagreements between reviewers were resolved by consensus. An

independent third reviewer was available to determine final data extraction in instances where consensus could not be reached. The appropriateness of pooling of data was determined based upon the degree of clinical and statistical heterogeneity between trials.

Result

A total number of 110 publications were included in this review. 55 studies were conducted in different countries (Table-1). Most of the studies were conducted in China.

14 were WHO reports, 37 were review articles and 5 were news reports. The objectives, type of patients, and the health care setting varied among studies making it difficult to generalize the results. Some studies were carried out under 'ideal' settings or in patient populations that are not representative of those presenting to health care facilities. Most of the studies were conducted in China (26). Other studies include populations from Hong Kong (11), Saudi Arabia (5), USA (3), Korea (2), Germany (3), Singapore (2) and one each from Canada, France and Oman.

Table 1: List of Studies

Author name	Country	Study design	Period of evaluation	Population	Laboratory test	Reference
Wang et al	China	Retrospective single centre case series	January 1 to Feb 3, 2020	138 hospitalized COVID-19 patients	rRT-PCR	15
Lu et al	China	Cross sectional	2019	9 COVID-19 patients	Next generation sequencing	20
Lau et al	Hong Kong	Cross sectional	2004-2005	59 horseshoe Bat	rRT-PCR, DNA sequencing, Viral culture, EIA, Genome sequencing	28
Nowotny et al	Oman	Cross sectional	2013	76 Dromedary camel	Specific serum IgG by protein microarray, Viral neutralization test	29
Lau et al	China	Cross sectional analytical	2017	1964 Bat samples	rRT-PCR, Viral culture	30
Ding et al	China	Cross sectional observational	2003	Autopsy samples of organs from 4 patients died of SARS and 4 control patients died of other disease	ICH, ISH, RT-PCR, Transmission electron microscopy	36
Oboho et al	Saudi Arabia	Descriptive analysis	2014	255 patients with laboratory confirmed MERS	Review of medical records	40
Alanazi et al	Saudi Arabia	Cross sectional	2014	Total 48 cases by contact tracing of MERS cases admitted into 2 hospitals	rRT-PCR , Genome sequencing	41
Lee et al	Hong Kong	Prospective	March 11 to 25, 2003	66 male,, 72 female SARS patients	rRT-PCR, Virus culture	42
Poissy et al	France	Prospective	2013	2 hospital admitted MERS patient	rRT-PCR	45
Bin et al	South Korea	Prospective	8 June 2015 to 3 July 2015	4 MERS-CoV patients in 2 hospitals	rRT-PCR, Virus isolation by cell culture	46
Chan et al	Hong Kong	Prospective	April,2003 to May 2004	hospitalized SARS patients	rRT-PCR	47
Guang et al	China	Retrospective	2003	a total 1,112 contactors of SARS	SARS-CoV Ab by neutralization test and ELISA method	48

Chan et al	China	Prospective	January 2020	5 patients in a family cluster with unexplained Pneumonia	rRT-PCR	49
Liu et al	China	Retrospective	January 2020	365 laboratory confirmed cases of COVID-19	rRT-PCR	50
Li et al	China	Retrospective	January 2020	1st 425 cases of confirmed novel coronavirus infected pneumonia (NCIP)	rRT-PCR	51
Huang et al	China	Prospective	January 2020	41 Hospital admitted confirmed cases of COVID-19	rRT-PCR	52
Rothe et al	Germany	Case report	January 2020	A 33 year old german man	rRT-PCR	55
Li et al	China	Prospective	Jan 31 to Feb 3	A Family cluster of 4 patients	r RT-PCR	57
Young et al	Singapore	Descriptive case series	Jan 23 to Feb 3	First 18 COVID-19 cases in 4 hospitals	rRT-PCR	59
Chang et al	China	Retrospective	Jan 28th to Feb 9th, 2020	16 confirmed COVID-19 patients	rRT-PCR	60
Booth et al	Canada	Cross sectional observational	March, 2003	environmental sample	rRT-PCR	62
Van Doremalen et al	USA	Cross sectional observational	2012	MERS-CoV (isolate HCoV-EMC/2012) strain studied	viral culture	63
Van Doremalen et al	USA	Cross sectional observational	January 2020	SARS-CoV-2 nCoV-WA1-2020 (MN985325.1) and SARS-CoV-1 Tor2 (AY274119.3) strain studied	viral culture	64
Ong et al	Singapore	Prospective	Jan 24 to Feb 4, 2020	3 confirmed hospitalised COVID-19 cases	rRT-PCR	67
Bin et al	South Korea	Prospective	8 June to 3 July 2015	4 MERS infected patients.	rRT-PCR viral culture	68
Xiao et al	Hong Kong	Cross sectional observational (Detailed modelling study/Multi agent model)	March 2003		bioinformatic analysis	69
Chan et al	Hong Kong	Cross sectional observational	2010	HKU39849 SARS-CoV viral strain	Virus culture	71
Duan et al	China	Cross sectional observational	2003	A total of 10(6) TCID50 viruses (SARS coronavirus strain CoV-P9) were placed in each tested condition	Virus culture	72
Rabenau et al	Germany	Cross sectional observational	2003	SARS-CoV isolate FFM-1 from	virus culture	73
Lai et al	Hong Kong	Prospective	SARS outbreak in 2003	a hospital admitted SARS patient	virus culture	74
Assiri	Saudi Arabia	Descriptive	1 September , 2012 to 15	47 laboratory confirmed cases of	rRT-PCR	78

			June, 2013	MERS		
Zing et al	China	Prospective	Jan 17 to March 6, 2020	3 paediatric cases of COVID-19	rRT-PCR	80
Holshue et al	China	Case report	January 2020	A 35 years old male COVID-19 case	rRT-PCR	81
Wu et al	China	Prospective	Jan 16 to March 15, 2020	98 hospital admitted confirmed cases of COVID-19	rRT-PCR	82
Wang et al	China	Prospective	Jan 1 to Feb 17, 2020	Hospital admitted confirmed COVID-19 cases	rRT-PCR	83
Zhang et al	China	Cross sectional study	Jan 2020	A hospital admitted laboratory confirmed case of COVID-19	rRT-PCR	84
Xu et al	China	Cross sectional analytical study	2019	Two public databases and single-cell transcriptomes from an independent data generated in-house.	Bioinformatics analysis	85
Alserehi et al	Saudi Arabia	Case report	2016	A 33 yr old ICU admitted MERS patient	rRT-PCR	87
Creanga et al	USA	Case control	May to June 2009	62 pregnant women with laboratory-confirmed 2009 H1N1 influenza	rRT-PCR	88
Lam et al	Hong Kong	Case control study	15 Feb to 31 May in 2003	10 pregnant SARS patient as case and 40 non pregnant SARS patient as control	rRT-PCR, SARS CoV Ab	90
Assiri	Saudi Arabia	Retrospective	Nov 2012 to Feb 2016	5 pregnant women with MERS	rRT-PCR	91
Wang et al	China	Prospective study	Jan 2020	Single COVID-19 patient pregnant women	rRT-PCR	93
Zhu et al	China	Retrospective analytical study	20 Jan to Feb 5, 2020	10 neonates born from diagnosed COVID-19 mothers	rRT-PCR	94
Fan et al	China	Prospective study	Feb 2020	2 cases of hospital admitted COVID-19 cases in 3rd trimester	Real time RT-PCR for 3 target genes of SARS-CoV-2	95
Chen et al	China	Retrospective review	Feb 2020	9 pregnant confirmed cases of COVID-19	rRT-PCR	96
Drosten et al	Germany	Case control	March 5 to March 27	18 suspected SARS patients	rRT-PCR sequencing	99
Grant et al	Hong Kong	Prospective	2003	26 hospitalized SARS patient	RT-PCR	100
Ng et al	Hong Kong	Prospective	March to May 2003	35 hospitalized confirmed SARS patients	rRT-PCR	101
Ng et al	Hong Kong	Prospective	March 13 to May 17, 2003	8 paediatric SARS patients	Real time RT PCR, SARS CoV IgG Ab	102
Huang et al	China	Prospective	January 2020	41 confirmed	rRT-PCR, Next	105

		study		hospital admitted COVID-19 cases	generation sequencing	
Chan et al	Hong Kong	Cross sectional study	January 2020	5 patients in a family cluster	point of care multiplex PCR	107
Xia et al	China	Prospective interventional case series study	Jan 26 to Feb 9	30 confirmed cases of nCoV pneumonia	rRT-PCR	109
Sun et al	China	Single centre cross sectional study	Dec 30 to Feb 7	hospital admitted COVID-19 patients	rRT-PCR	110
Xu et al	China	Cross sectional non randomized	January 2020	Diagnosed and suspected cases of COVID 19	rRT-PCR	111

Table 2. Stability of SARS-CoV-2, SARS-CoV and MERS-CoV on different environmental surface

Types of surface	Virus	Strain	Load applied	Temperature	Persistence	Reference
Plastic	SARS-CoV-2	nCoV-WA1-2020(MN985325.1)	$10^{5.25}$	Not specified	72h	64
	SARS-CoV	Tor 2(AY274119.3)	$10^{6.75-7}$	22-25	≤ 5d	64
	SARS-CoV	Strain HKU39849	10^5	20°C	48h	71
	MERS-CoV	Isolated HCoV-EMC/2012	10^5	30°C	8-24h	63
Stainless Steel	SARS-CoV-2	nCoV-WA1-2020(MN985325.1)	$10^{5.25}$	Not specified	48h	64
	SARS-CoV	Tor 2(AY274119.3)	$10^{6.75-7}$	20°C	48h	64
	MERS-CoV	Isolated HCoV-EMC/2012	10^5	30°C	8-24h	63
Copper	SARS-CoV-2	nCoV-WA1-2020(MN985325.1)	$10^{5.25}$	Not specified	4h	64
	SARS-CoV	Tor 2(AY274119.3)	$10^{6.75-7}$		8h	64
Cardboard	SARS-CoV-2	nCoV-WA1-2020(MN985325.1)	$10^{5.25}$	Not specified	24h	64
	SARS-CoV	Tor 2(AY274119.3)	$10^{6.75-7}$		8h	64
Glass	SARS-CoV	Strain P9	10^5	RT	4d	72
Metal	SARS-CoV	Strain P9	10^5	RT	5d	72
Wood	SARS-CoV	Strain P9	10^5	RT	4d	72
Polystyrene Petri Dish	SARS-CoV		10^{6-7}	RT	> 6d	73
Paper	SARS-CoV	Strain P9	10^5	RT	4d	72
	SARS-CoV	Strain GVU6109	10^6	RT	24h	
			10^5		3h	74
Disposable Gown	SARS-CoV	Strain GVU6109	10^6	RT	2d	74
			10^5		24h	

RT= Room Temperature

Reservoir and Animal to Human Transmission

In order to develop preventive strategies to contain the infection it is fundamental to determine the host of origination and route of transmission. In December, 2019, clusters of atypical pneumonia with unknown etiology were identified in Wuhan city of China which was epidemiologically linked to a seafood wholesaler market¹⁸. Virus isolation from patients and rapid sequence analysis revealed that the pathogen is a novel beta corona virus which showed 96.2% overall genome sequence homology with Bat CoV RaTG13, 79.6% similarity with SARS-CoV¹⁹ and only 50% identity to MERS-CoV²⁰. Different studies have demonstrated that bats harbor a wide variety of CoVs including SARS related-CoV and MERS related-CoV suggesting that immediate ancestor SARS-CoV-2 has been circulating in bats for several years. It requires further confirmation

whether SARS-CoV-2 is directly transmitted from bat to human or through an intermediate host²¹. Several other animals like turtles, pangolin snakes and many species showed sequence homology of receptor residues of SARS-CoV-2 which strengthens the possibility of presence of an intermediate host. So far, the intermediate hosts of SARS-CoV-2 are elusive and pangolins, snakes, minks are reported to be the plausible host²². Recently, a research group of South China Agricultural University analyzed more than 1,000 metagenomic samples and reported that 70% of pangolins were positive for the coronavirus²³. Sequence analysis of the strain found in pangolin showed 97% sequence homology in the Receptor Bindings Domain (RBD) with the current infected human strain SARS-CoV-2²⁴. Another group of researchers observed that a unique peptide (PRRA) insertion is present in the human SARS-CoV-2 virus, which is absent in pangolin originated strain and they

concluded that SARS-CoV-2 may not be directly come from pangolin to human host²⁵. To determine what this intermediate host species might be it is imperative to perform far wider sampling of animals from wet markets or that live close to human populations. In case of SARS-CoV, phylogenetic analysis showed that it is a beta corona virus and closely related to the CoVs found in civets, raccoon dogs, ferret badgers and Chinese horseshoe bats (*Rhinolophus sinicus*) in Guangdong Province of South China where initial epidemic was breaking out in 2002²⁶. Researchers initially thought that civets or raccoon dogs are the primary reservoir hosts which are sold in Chinese wet market as a food source. Later on, the Chinese horseshoe bat appears to be the natural reservoir of the ancestral SARS-CoV, because the mutation rates of different genes of the strains found in bats were low, while rapidly evolving process of adaptation of a viral strain in animals evident by high mutation rate were observed in those of the civet strains^{27,28}. It may indicate an interspecies transmission from bats to intermediate hosts like civets and finally to humans. MERS-CoV is another highly pathogenic zoonotic CoV that was identified in Saudi Arabia in 2012. MERS-CoV-like virus was identified in *Pipistrellus* and *Perimyotis* bats suggesting that bats are the potential reservoir and transmitting host. Studies have shown that dromedary camel MERS-CoV strains are almost identical to human MERS-CoV, proffering that MERS-CoV was transmitted from bat to camel, an intermediate host then to terminal human host²⁹. Sequence analysis revealed that strain of MERS-CoV found in bat had 60-70% homology to camel and human MERS-CoV postulated that it had to undergo several amino acid changes in receptor binding protein to become capable of infecting camel and human host³⁰⁻³¹.

Human to Human Transmission

Sustained human to human transmission is necessary to establish a transmission cycle and to initiate an outbreak. An efficient human to human transmission requires the ability of the virus to attach to human cells. Corona viruses attach to the host cell via spike protein³². Human angiotensin converting enzyme 2 (ACE-2) is shown to be the receptor of SARS-CoV-2 and SARS-CoV,³³ whereas MERS-CoV uses dipeptidyl peptidase 4 (DPP-4) receptor to enter into host cell³⁴. Apparently SARS-CoV-2, MERS-CoV and SARS-CoV all three highly pathogenic corona viruses spread efficiently using multiple routes among humans. Airborne transmission via droplets and contact with contaminated surfaces are the accepted transmission route, whereas aerosol transmission, feco-oral transmission, perinatal, blood borne transmission are the plausible routes of transmission of highly pathogenic CoVs³⁵⁻³⁷.

Respiratory Droplet Transmission

Direct contact transmission via respiratory droplet is the most frequent and established route of transmission of respiratory viruses where droplets (> 5 µm diameter, travelling < 1 m) containing viable viruses may produce

during expiratory activities like talking, coughing, sneezing and laughing. This droplet may come in contact with the nose, mouth, eyes, or upper respiratory tract of susceptible individuals who come in close proximity to the infected person and eventually they become infected³⁸. Droplet transmission appears to be a major route of transmission of SARS-CoV and MERS-CoV. Primarily cases were detected in household and hospital settings in persons who remain in close contact with the SARS-CoV and MERS-CoV infected patients³⁹⁻⁴³. Occasional transmission also documented among casual and social contacts after prolonged and intense exposure to a SARS-CoV case⁴⁴. Both upper (nasopharyngeal and oropharyngeal swab) and lower (sputum, tracheal aspirate, and bronchoalveolar lavage fluid) respiratory tract samples gave positive results for MERS-CoV RNA and live virus. Prolonged period of viral shedding through respiratory route was also documented during MERS-CoV outbreak which was evident by detection of viral RNA for more than 1 months and live virus up to 25 days in a few cases after onset of symptoms⁴⁵⁻⁴⁶.

SARS-CoV RNA in respiratory samples of infected patients became detectable within 4 days, gradually increasing from 6 to 11 days of symptom onset and started to decline after 16 days but in some patient RNA was detectable for >30 days whereas virus could be isolated up to first 2 weeks of the illness and generally become negative after 3 weeks⁴⁷. SARS-CoV and MERS-CoV infected patients remain infectious during their symptomatic period. Transmission of SARS-CoV and MERS-CoV during incubation period or from asymptomatic patients was not reported till today^{37,48}. Up to present, all the evidence indicates that droplet transmission among close contacts is the main transmission route of SARS-CoV-2⁴⁹⁻⁵². Four generations of spread (first case acquired this infection from a nonhuman source who infected someone else, who infected another individual, who then infected another individual) of SARS-CoV-2 was identified suggesting that sustained human to human transmission cycle has already been established⁵³⁻⁵⁴. SARS-CoV infected patients may transmit the virus during their early phase of infection and asymptomatic carriers also seem to be infectious⁵⁵⁻⁵⁷.

SARS-CoV-2 RNA was detected from upper respiratory tract samples prior 21-2 days of symptoms onset and continue to persist for 7 to 12 days and in some severe cases up to 2 weeks⁵⁸. In Singapore prolonged persistence of viral shedding, at least for 24 days in nasopharyngeal swab was recorded⁵⁹. Researchers observed that Virus continues to shed up to 8 days in respiratory samples after apparent resolution of symptoms⁶⁰. So far, it is not clearly evident how long SARS-CoV infected patients remain infectious.

Aerosol Transmission

Aerosols also known as 'droplet nuclei' which are small particles of ≤ 5 µm diameter and can travel > 1m, usually produced through the process of rapid drying up of

exhaled respiratory droplets³⁸. Aerosol transmissible infectious agents have two key features a) their tendency to follow air flows and remain suspended in the air for prolonged period of time which facilitates onwards transmission and b) their small size, which requires an enhanced filtering capacity. Moreover, it demands costly PPE (personal protective equipment) of healthcare providers that can tightly seal the airways while providing patients care or performing aerosol generating procedures⁶¹. It is necessary to find out whether highly pathogenic CoVs are aerosol transmissible or not as route of transmission has significant implications on containment strategies of these infectious agents. Previous data regarding transmission of SARS-CoV and MERS-CoV suggested that major route of transmission was respiratory droplet and close contact but aerosol transmission may have played some role in nosocomial and super spreading events during outbreak⁶¹⁻⁶³. It is still controversial whether SARS-CoV-2 transmitted through aerosol or not besides respiratory droplet. In a recent study, aerosol containing SARS-CoV-2 and SARS-CoV were generated with the use of a three jet collision nebulizer and fed into a Goldberg drum to create an artificial aerosolized environment. Both SARS-CoV-2 and SARS-CoV remained viable up to 3 hours in the suspended air suggesting that these highly pathogenic viruses may have the potential to be transmitted through aerosol and higher chances of nosocomial infection⁶⁴. WHO recommended “airborne precautions” for healthcare workers in specific circumstances that generate aerosol (i.e. endotracheal intubation, bronchoscopy, open suctioning, administration of nebulized treatment, manual ventilation before intubation, turning the patient to the prone position, disconnecting the patient from the ventilator, non-invasive positive-pressure ventilation, tracheostomy, and cardiopulmonary resuscitation) though previous experiment that was conducted in artificial environment doesn't reflect normal human cough condition or clinical settings⁶⁵. Besides that, different environmental condition such as UV light, humidity, temperature, also affect the viability of infectious agents which were not considered in recent study⁶⁶. Another study demonstrated the presence of SARS-CoV-2 RNA in swab taken from the air exhaust outlets collected from COVID-19 positive patient's room before routine cleaning, suggested that virus containing small air droplets may be deposited though they did not observe the viability of virus. They also examined the SARS-CoV-2 RNA in air samples of that patient's room and all the air samples showed negative results for viral RNA⁶⁷. Although there is not clear evident of aerosol transmission, but it should be considered as a potential route of transmission at least in special settings where aerosol generating procedures are performed and proper precautions should be adopted.

Indirect Contact Transmission

Inanimate objects that can be contaminated by infectious agents are known as fomites that serve as a medium for transfer of organisms between hosts. Indirect contact transmission is another major route of transmission of

SARS-CoV-2, SARS-CoV and MERS-CoV viruses. Infectious droplets of varying sizes are produced by breathing, coughing or sneezing of CoV infected patients and settled down onto different surfaces (fomites). When someone touches this contaminated surfaces, they can be infected by self-inoculating the coronaviruses into mucosal membranes like in the eyes, nose and mouth. In healthcare settings frequent touch surfaces act as a potential source of infection^{63,67-69}. Several factors determine the potentiality of spread of CoVs through fomites such as stability of corona viruses on such surfaces, viral load, environmental condition and the infective susceptibility of the different exposed tissues of host. Analysis of several studies reveals that SARS-CoV and MERS-CoV have unusual capacity to survive on different inanimate surfaces for several hours to days like non-enveloped viruses⁷⁰. Higher temperature reduces the duration of persistence of MERS-CoV whereas increase viral load increases the stability of SARS-CoV and MERS-CoV^{63,71}. MERS-CoV detected on steel and plastic after 48 hours at 20°C whereas at 30°C viability reduced to 8 to 24 hours depending on humidity⁶³. Currently a comparative study was conducted under laboratory condition between SARS-CoV-2 and SARS-CoV showed that stability of both viruses is almost similar on plastic, stainless steel, copper and cardboard. Highest viability of SARS-CoV-2 observed on plastic that was 72 hours, on steel 48 hours, whereas no viable SARS-CoV-2 was found on cardboard after 24 hours⁶⁴. SARS-CoV remains viable on polystyrene petri dish more than 6 days and on disposable gown from 5 min to 2 days depending on viral load⁷³⁻⁷⁴. So far there is no scientific study examined the viability of SARS-CoV-2 and MERS-CoV on fabrics. Porous fabrics like cardboard where SARS-CoV-2 survives for 24 hours, so it may remain viable on porous fabrics for at least 24 hours. But zipper, button which is made of steel, SARS-CoV, MARS-CoV and SARS-CoV-2 may survive for longer time. expertise suggested that polyester, spandex may retain virus more time than cotton fabrics as cotton fabrics are more porous which is beneficial. Porous fabrics can trap the virus containing droplets and suck out fluid which leads to loss of infectivity of virus⁷⁵. Dr. Schmidt also warns that coronaviruses may survive on shoes and synthetic fabrics for 5 days though no study was published where viability of SARS-CoV-2 on shoes were examined till today⁷⁶. Findings of different studies (Table-2) and valuable opinions of expertise highlighted that there is a higher chance of indirect contact transmission of SARS-CoV-2 via fomites.

Feco-oral Transmission

SARS-CoV and MERS-CoV have been reported to cause alimentary symptoms and presence of viral RNA was also detected in previous studies⁷⁷⁻⁷⁸. During the SARS outbreak back in 2002-2003, a study revealed presence of SARS-CoV RNA in patients' fecal specimens from the fifth day of disease which continued to rise and peaked at the 11th day. In a minor number of patients the viral RNA remained positive even after 30 days of disease onset⁴⁷. A study published in 2016 by Corman⁷⁹ shows

that 14.6% of fecal specimens from MERS-CoV infected patients were positive for viral RNA. Prolonged presence of SARS-CoV and MERS-CoV in fecal samples and their ability to withstand the various environmental conditions may facilitate their feco-oral transmission⁷¹. Till date, reports from Wuhan stated that 2 to 10% of COVID-19 patients have shown enteric symptoms like abdominal pain, diarrhoea and vomiting and patients who required ICU support presented with these symptoms more. Moreover, stool sample collected from confirmed cases of SARS-COV-2 infection in Wuhan China shows presence of viral RNA¹⁵. Prolonged shedding of SARS-CoV-2 RNA was reported among pediatric children and it was observed that their throat swab became negative after 2 weeks of fever abatement whereas viral RNA remained persistent for 1-3 weeks after throat swab became negative⁸⁰. The first patient diagnosed with COVID-19 in the United States was also checked for SARS-COV-2 RNA in his fecal sample which showed similar results⁸¹. Another study demonstrated that fecal specimens continued to be SARS-CoV-2 RNA positive for a mean of 11.2 days more than respiratory swab⁸². Importantly live virus has reportedly been cultured from stool samples besides viral RNA from COVID-19 patients^{83,84}. Another study has revealed that expression of ACE-2 receptor in the oral cavity may facilitate the entry of SARS-CoV-2⁸⁵. Meanwhile it is quite obvious that SARS-COV-2 can live longer in the alimentary tract of the patients than the respiratory tract strongly suggests that there is a possibility of fecal oral transmission. Ingestion of food contaminated with viable virus or inhalation of droplets containing virus from stool is the suggested possible way of transmission from faces although the chance of transmission and infection by ingestion or inhalation need much more evaluation^{54, 81}.

Perinatal Transmission

It has been evident by previous studies that pregnant women are very much susceptible to viral infections including SARS-CoV and MERS-CoV which accounts for adverse pregnancy outcomes like increased mortality rate of pregnant women, miscarriage, preterm delivery, intrauterine growth retardation of fetus⁸⁶⁻⁸⁹. In spite of such adverse outcome of viral infection during pregnancy, no documentation of perinatal transmission of SARS-CoV and MERS-CoV from mother to fetus was found till date^{87,90-91}. During the outbreak of SARS-CoV infection in 2002 RT-PCR and viral cultures of blood, cerebrospinal fluid, respiratory secretions, excreta, and other body fluids of newborns from SARS infected mother showed negative results for virus⁹². Very limited numbers of studies were conducted so far where transmissions of SARS-CoV-2 from mother to child in different potential routes were evaluated. A 28-year-old pregnant lady was diagnosed with COVID-19 at Suzhou Municipal Hospital who eventually underwent an emergency Cesarean Section at 30 weeks of gestation. Samples of placenta, umbilical cord blood, amniotic fluid, gastric juice and throat swab of the infant were obtained during the delivery and all the samples tested

negative for SARS-CoV-2 RNA. Throat swab and stool specimen collected after 3 days of delivery showed the same result⁹³. Huaping-Zhu⁹⁴ also revealed similar negative results for SARS-CoV-2 nucleic acid in pharyngeal swabs collected from 9 neonates born from confirmed cases of COVID-19⁹⁴. Cuifang and colleagues⁹⁵ reported two cases of COVID-19 positive pregnancy during the third trimester. Presence of SARS-CoV-2 could not be detected by serial qRT-PCR in the products of conception and the neonates which supports the previous idea of limited possibility of the viral transmission through placenta or via ingestion of cervicovaginal fluid⁹⁵. A retrospective study was conducted by Chen and colleagues⁹⁶ on 9 laboratory confirmed cases of COVID-19 pneumonia in Zhongnan Hospital of Wuhan University. From all the 9 live births, specimens of 6 newborns were tested and found negative for viral nucleic acid which indicates no transplacental entry into the fetus⁹⁶. Despite all the evidence not in favour of perinatal transmission of SARS-CoV-2, some different scenarios demand attention. During the recent outbreak of SARS-CoV-2 infection in 2020, presence of viral RNA is detected after 30 hours of delivery in a pharyngeal swab obtained from a newborn of a confirmed case COVID-19 in Wuhan, China. This incident points to the possible risk of vertical transmission of the virus⁹⁷. A similar case reported in Tongji Hospital suggests that the chance of vertical transmission of SARS-COV-2 is yet to be evaluated as definitive in a large scale⁹⁸. Scarce information about clinical features and transmission of SARS-CoV-2 infection during pregnancy renders the necessity of further studies so that necessary preventive measures can be taken.

Transmission via Transfusion

As SARS-CoV-2 is highly infectious and spreading faster across the world hence every possible route of transmission should be considered. Though viral RNA was detectable in serum or plasma samples of patients infected with both in SARS-CoV and MERS-CoV^{79,99,100}, but no transmission by blood or other substance of human origin have been documented to date¹⁰¹. In adult cases SARS-CoV RNA was detected in 78.0% cases during the first week of illness¹⁰². Another study on pediatric SARS patients found out viremia in 87.5% of the cases¹⁰³. Viral shedding in plasma was found to be common during the clinical course of SARS-CoV infection in 2003 and 79.0% cases became positive within 3 days of onset of fever with a peak rise around day 4-5, after which it was diminished¹⁰². Study on theoretical estimated risk of SARS-CoV transmission through blood transfusion revealed that the mean risk was 14.11 per million with maximum risk 23.57 per million¹⁰⁴. MERS-CoV infection is relatively more fatal and causes highly lethal respiratory distress as that of SARS⁷⁹. In a study it was observed that high viral load was detected in serum samples within the first week of diagnosis of MERS-CoV infection in some cases¹⁰⁵. Similarly, SARS-CoV-2 infection also showed positive results for viral RNA in plasma or serum samples¹⁰⁶. The

infection caused SARS-CoV-2 is mainly diagnosed by the presence of viral RNA in the pharyngeal swab but viral nucleic acid has also been detected in the plasma of COVID-19 patients¹⁰⁶. The first 41 confirmed cases of COVID-19 in Wuhan, China were investigated by Huang and colleagues and in 15.0% (6/41) cases viremia was detected. This study also revealed a very low plasma concentration of SARS-CoV-2 RNA both in case of patients under intensive care unit and patients having mild symptoms¹⁰⁶. In another study, during this recent outbreak, 1 of 6 cases was found to have weak positive results for viral nucleic acid¹⁰⁷. As there is so much yet to be discovered about the SARS-CoV-2 in every aspect and asymptomatic cases also account for the possibility of transmission¹⁰⁸, it will be wise to reconsider the blood products as a potential source of infection according to the findings till now.

Transmission via Conjunctiva

Based on a recent case report and evidence of virus detection from the eye among cases with conjunctivitis, ocular surface is thought to be another possible route of transmission of SARS-CoV-2. A healthcare worker became infected with SARS-CoV-2 after visiting a patient wearing an N95 respirator, but no eye protection. Among 30 confirmed COVID-19 patients only one patient showed positive results for viral RNA in his tear and conjunctiva secretion¹⁰⁹. In a cross-sectional study of 72 laboratory-confirmed cases of COVID-19, two patients (2.8%) had conjunctivitis. Viral RNA fragments were identified in ocular discharges using a RT-PCR assay in one patient with conjunctivitis¹¹⁰. In another cross-sectional study, RT-PCR assays of conjunctival secretion were conducted on 14 confirmed cases and 16 suspect cases of COVID-19. All results were negative¹¹¹. Ophthalmologist should be aware and take protective measure while providing care to a patient presented with conjunctivitis because ophthalmologists may be the first providers to evaluate patients possibly infected with COVID-19.

Discussion

The systematic review incorporated latest highly informative studies and evidence regarding established and potential routes of SARS-CoV-2 transmission. It also highlighted transmission of SARS-CoV and MERS-CoV which caused outbreaks in the past. As SARS-CoV-2 is highly infectious and spreading faster across the world hence every possible route of transmission should be considered.

Since the first reports of SARS-CoV-2 in the seafood market of Wuhan, China, there has been considerable discussion on the origin of the causative virus. Genome sequence analysis firmly determines that it originated from Bat, though it is still controversial whether an intermediate host exists or not like SAR-CoV and MERS-CoV^{19,20,21}. So far, coronavirus identified in pangolin showed the highest level of similarities with

human originated SARS-CoV highlighting its potential to act as an intermediate host^{24,25}.

Transmission of SARS-CoV-2 has been documented to occur frequently and inevitably via respiratory droplets as the viable virus comes out from the infected person through expiratory activities and infects another person by direct contact. Sustained human to human transmission cycle has been established by detecting four generation spread of SARS-CoV-2. Moreover, detection of the viral RNA in the respiratory tract prior to the onset of symptoms and days after recovery makes this established route the prime one to consider^{51,52,53,54}.

One study revealed that SARS-CoV-2 remained viable in the air for 3 hours after aerosol generating procedure in artificial settings⁶⁵, whereas viral RNA was detected in air exhaust outlets of COVID-19 positives patients' rooms⁶⁷. SARS-CoV and MERS-CoV also found to be persistent in the air after aerosol generating procedure suggested that at least in some instances where aerosol is generated, airborne precautions should be adopted to prevent transmission of SARS-CoV-2 (Table-2).

Studies conducted on survivability of SARS-CoV-2 on different surfaces confirmed that contaminated fomites are potential route of transmission of SARS-CoV-2 (Table-2). Therefore, hand washing before touching the mouth, nose and eyes after contact with contaminated surface is highly recommended.

The possibility of SARS-CoV-2 transmission through feco-oral route gained considerable attention as the fecal samples collected from patients of different ages and countries were positive for viral RNA as well as viable virus during the current outbreak^{15,83,84}. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 showed longer viability in the alimentary tract than the respiratory tract^{80,81}. A study suggested that expression of ACE-2 receptor in the oral cavity may facilitate the entry of SARS-CoV-2 which points to the strong potential of this route of transmission⁸⁵.

Although no significant perinatal transmission of SARS-CoV and MERS-CoV from mother to fetus was documented till date, these two viruses were found to cause several negative pregnancy outcomes^{86,87,88,89,90,91}. The current COVID-19 outbreak allowed very few studies to be conducted regarding this issue. Most of them reported absence of viral RNA in products of conception as well as neonatal pharyngeal swab which suggest no transplacental entry of virus^{93,94,95,96}. In contrast, we found two case reports from China where viral RNA was detected in the pharyngeal swab of the neonate^{97,98}. Scarce information about perinatal transmission renders the necessity of further studies so that necessary preventive measures can be adopted.

Serum or plasma samples of patients infected with SARS-CoV and MERS-CoV have tested positive for viral RNA in many cases but transmission via blood

products has not been documented so far^{79,99,100,101,102,103,104,105}. We observed more or less similar picture while reviewing the studies of the recent COVID-19 outbreak. SARS-CoV-2 infection is mainly diagnosed by presence of viral RNA in the respiratory tract samples but few reports from China showed that it has also been detected in some patient's serum or plasma^{106,107,108}. As there is so much yet to be discovered about the SARS-CoV-2 in every aspect and asymptomatic cases cannot be completely spared from the possibility of transmission, it will be wise to reconsider the blood products as a potential source of infection.

Although recent studies revealed that viral RNA was detected in ocular secretions of very few COVID-19 patients and conjunctivitis was a rare clinical feature of current outbreaks^{109,110,111}, ophthalmologists should consider the ocular surface as a potential route of transmission of SARS-CoV-2 and take necessary precautions while attending patients presented with conjunctivitis.

Conclusion

Until now, no promising clinical treatments or preventive strategies have been developed against newly emerging novel SARS-CoV-2. In this regard interruption of sustained human to human transmission is the best possible way to combat this deadly virus. Clear understanding of transmission routes and infectivity is essential so that appropriate protective measures can be adopted to prevent its spread. Epidemiologic observations and laboratory studies suggested that SARS-CoV-2 is highly infectious and mainly transmitted by close contact probably via droplets and contaminated fomites, but transmission via aerosols, feco-oral and other potential route should take into consideration in this current situation and appropriate protective measures should be employed to overcome the pandemic as soon as possible.

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