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Nosocomial outbreak of the Middle East Respiratory Syndrome coronavirus: A phylogenetic, epidemiological, clinical and infection control analysis

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Dear Prof. Pat,

All authors contributed equally

Ziad

Title:

Nosocomial Outbreak of the Middle East Respiratory Syndrome Coronavirus: A Phylogenetic, Epidemiological, Clinical and Infection Control Analysis

Running title:

MERS nosocomial outbreak – a phylogenetic analysis

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ABSTRACT

Background: Middle East Respiratory Syndrome coronavirus (MERS-CoV) continues to cause intermittent community and nosocomial outbreaks. Obtaining data on specific source(s) and transmission dynamics of MERS-CoV during nosocomial outbreaks has been challenging. We performed a clinical, epidemiological and phylogenetic investigation of an outbreak of MERS-CoV at a University Hospital in Riyadh, Kingdom of Saudi Arabia.

Methods: Clinical, epidemiological and infection control data were obtained from patients and Healthcare workers (HCWs). Full genome sequencing was conducted on nucleic acid extracted directly from MERS-CoV PCR-confirmed clinical samples and phylogenetic analysis performed. Phylogenetic analysis combined with published MERS-CoV genomes was performed. HCWs compliance with infection control practices was also assessed.

Results: Of 235 persons investigated, there were 23 laboratory confirmed MERS cases, 10 were inpatients and 13 HCWs. Eight of 10 MERS inpatients died (80% mortality). There were no deaths among HCWs. The primary index case assumed from epidemiological investigation was not substantiated phylogenetically. 17/18 of MERS cases were linked both phylogenetically and epidemiologically. One asymptomatic HCW yielded a MERS-CoV genome not directly linked to any other case in the investigation. Five HCWs with mild symptoms yielded >75% full MERS-CoV genome sequences. HCW compliance with use of gowns was 62.1%, gloves 69.7%, and masks 57.6%.

Conclusions: Several factors and sources, including a HCW MERS-CoV 'carrier phenomenon', occur during nosocomial MERS-CoV outbreaks. Phylogenetic analyses

of MERS-CoV linked to clinical and epidemiological information is essential for outbreak investigation. The specific role of apparently healthy HCWs in causing nosocomial outbreaks requires further definition.

Keywords: Middle East respiratory Syndrome, Nosocomial, Outbreak, Phylogenetics, Epidemiology, Healthcare workers, MERS-CoV carrier phenomenon

INTRODUCTION

The Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV) (1) is listed in the 2019 WHO Blueprint priority list of pathogens (2) because it causes high mortality rates in humans(3), there are currently no specific treatments or vaccines and it remains a threat to global health security. Since the first identification of the MERS-CoV as a novel zoonotic human pathogen in September 2012 (4), it continues to circulate in the Middle East causing intermittent community and healthcare associated outbreaks, as well as in returning travelers from the Middle East (5)As of January 10th, 2020, a total of 2468 laboratory-confirmed cases of MERS-CoV infection, with 851 deaths (34.5% mortality) were reported from 27 countries to the WHO, the majority (2073 cases, 772 deaths) occurred in the Kingdom of Saudi Arabia (KSA) (1). Health care associated outbreaks of MERS-CoV are a hallmark of MERS-CoV and they account for approximately 40% of MERS cases reported to date Large outbreaks have occurred in KSA (1,6-12) and the largest outside KSA occurred in the Republic of Korea (South Korea) in 2015 (1,13,14).

Phylogenetic analysis of MERS-CoV strains aligned to epidemiological and clinical information is important for identifying the index case, source(s) of transmission, transmission patterns, surveillance and evolution of MERS-CoV genomes (6,7,9,13). Genomic sequencing of MERS-CoV and molecular epidemiology can reveal spatiotemporal patterns that help identify whether all MERS-CoV infections originated from a single or multiple source(s), with subsequent human-to-human transmission, or from several sources. The focus of nosocomial outbreaks is usually on instituting infection control measures, identification of the primary MERS case, preventing further nosocomial spread between patients and healthcare workers (15). Whilst clinical and epidemiological information are usually available from outbreak response, obtaining phylogenetic information remains challenging and has not been forthcoming from KSA since 2015. In a review by Grant *et.al*. the prevalence of asymptomatic and mildly symptomatic MERS amongst Health Care Workers (HCW) was 11% and 26% respectively (16). The possible role of mildly symptomatic or asymptomatic MERS-CoV-infected healthcare workers as 'carriers' of MERS-CoV has been highlighted and needs further investigation (15-19).

We performed a clinical, epidemiological, phylogenetic and infection control practices investigation of a large nosocomial outbreak of MERS-CoV at King Khalid University Hospital (KKUH), Riyadh, KSA.

METHODS:

***Study site*:** King Khalid University Hospital (KKUH), Riyadh, Saudi Arabia, an 850-bed primary, secondary and tertiary care facility with all general and subspecialty medical services with three intensive care units (ICU) twelve inpatient wards including a cardiology/cardiac surgery ward, a haemodialysis unit (HD) and an emergency room (ER) which has three different units, including a resuscitation unit (RU) with ten beds that is only equipped with one single airborne infection isolation room (AIIR), the remainder beds are separated by curtains. Intervention was part of the standard of care for hospital outbreak management and individual oral consent for nasopharyngeal swabs (NPS) was standard. The study was approved by the hospital's Institutional Review Board (IRB) number: E-15-1464.

***Study population, timelines and MERS-CoV case detection*: Study design was a prospective surveillance study for all suspected** Patients and healthcare workers (HCW) to be infected with MERS-CoV during a hospital outbreak that spanned over a forty-five days period from early February till mid-March 2015.

After identifying the first case, the hospital MERS-CoV infection control outbreak team was activated and followed the national MERS-CoV action plan.

Viral genome sequencing and phylogenetic analysis:

MERS-CoV PCR-positive samples were subjected to next generation sequencing (NGS) using established methods (20-25). Briefly, clinical samples were screened with RT-PCR, with amplification targeting both the upE and ORF1A for confirmation. NGS

was performed on nucleic acid extracted from real-time PCR confirmed cases of MERS-CoV. 50 µL of nucleic acid was generated from 200 µL of tracheal aspirate or from a nasopharyngeal or throat swab with automated processing. PCR amplification of DNA amplicons covering the entire MERS-CoV genome were prepared. The PCR amplicons for each sample were pooled for Illumina library (Illumina, San Diego, CA, USA) preparation with each sample processed to include a unique barcode sequence. Standard MiSeq 150nt paired-end reads were generated. Sequence data were de-multiplexed into sample-specific readsets, processed to remove adapter and primer sequences at the ends of reads, and trimmed from their 3′ end until the median Phred quality score was >35, discarding reads smaller than 125 nucleotides using QUASR (22). The processed readsets were *de novo* assembled into large contiguous sequences (contigs) using SPAdes v.3.13.0 (23). Final quality control of genomes included checking intactness of open reading frames (ORFs) for full genomes, comparison of the obtained sequences and the encoded proteins with reference sequences retrieved from GenBank. All single nucleotide polymorphisms in the outbreak set were verified by counting all quality controlled short reads mapping across the position. A total of 15 samples yielded >80% of the 30119nt MERS-CoV genome and 18 samples yielded >50% genomes which were examined in detail. The 18 assembled genomes were aligned using MAFFT v.7.42 (24) and manually checked in Aliview. (25), and the both ends were trimmed to the longest shared sequence (final length 30,123nt). As previously described (26.27), a Bayesian phylogenetic tree was inferred using MrBayes v.3.2.7a (28) under the best fitted model of substitution estimated in IQTREE (29,30), run in duplicate with 1 million generations with sampling performed

every 1,000 generations and with a removal of 25% burn-in. Three independent chains were run and checked for chain convergence.

Compliance with infection control measures:

A questionnaire and interview study were performed at end of outbreak of a random selection of 68 HCWs contacts of all MERS patients (35 from ER, 12 from ICU, 10 from cardiac ward, 6 other) to assess compliance with infection control practices.

RESULTS:

Demographic, epidemiological and clinical characteristics:

A total of 23 laboratory confirmed MERS cases were diagnosed during the outbreak: 10 were patients and 13 healthcare workers. The description of the outbreak is described in terms of Cases # and HCW # in respect to chronological diagnosis:

The first identified MERS case (Case #1) was a male gentleman in his 40s- who presented to an outside hospital with acute myocardial infarction, he was transferred to our institution for coronary artery bypass grafting. On the first post-operative day he was extubated and during the ensuing days he mobilized well and socialized with other patients in neighbouring rooms in the cardiac surgery ward including patients who were later identified as Case #2 and Case #3.

On the 4th post-operative day Case #1 developed fever, chest pain, shortness of breath (SOB), and was diagnosed with pneumonia and a MERS-CoV PCR test from a respiratory sample returned to be positive. He was transferred to critical care unit

where he died four weeks later, he was identified epidemiologically as the index case, in the meantime Case #2 was discharged home before onset of symptoms, only to return to ER nine days later with fever and SOB, he was placed in RU without AIIR adjacent to Case #4 who was already in RU for an upper gastrointestinal bleed, nine days later she developed SOB and fever . Case #3 who was still in cardiac surgery ward at the same time developed fever and SOB and was transferred to ICU, all three new cases nasopharyngeal swabs (NPS) tested positive by PCR for MERS-CoV, and all died. First HCW identified be infected (HCW#1) developed fever and cough two days after caring for Case #4 in RU. Case #5 was placed in RU between Case #2 and Case #4 in a "disaster bed" without any barrier due to an overwhelmingly busy ER and was transferred to cardiac ward prior to onset of respiratory symptoms that developed ten days later in the form of cough, he ultimately recovered, while both other two cases died. Second, third, fourth and fifth infected HCWs (HCW #2, HCW #3, HCW #4 and HCW #5) cared for both Case #2 and Case #4 and were commonly mingling with HCW #1. The 6th HCW (HCW #6) did NPS for Case #4 without personal protective equipment (PPE). Case #6 was in a common room in cardiac ward adjacent to Case #5. HCW #7 and HCW #8 worked in RU and cared for Case #2. Case #7 was diagnosed in a separate ward and was not linked epidemiologically to any of the previous cases or HCWs, she died. HCW# 9 worked in RU and cared for Case #4. HCW# 10 was in direct contact with HCW #3. Case #8 was in also in a common room with Case #5 and Case #6, both Case#8 and Case#6 died. HCW #11 was also in contact with Case #4. HCW #12 was in contact with HCW#1 and was asymptomatic only detected by contact tracing. Case #9 was admitted in a common room adjacent to Case #7, and ultimately recovered. HCW #13 intubated Case #6 without PPE. Case #10 was admitted in a

common room adjacent to Case #5. The outbreak primarily affected RU in ER and Cardiac ward and was declared clear 14 days after the death of Case #10.

Eleven of the thirteen HCWs were symptomatic with only mild symptoms, one was totally asymptomatic who was detected by contact tracing, and one had severe disease that required ICU admission but ultimately recovered. 225 HCWs who were the total staff working in RU, ICU and cardiac ward were screened for MERS-CoV regardless of their contact history with cases or infected HCWs. Of the total 23 MERS-CoV infected individuals, 8 died (overall mortality 35%). Of the 10 patients with MERS-CoV infection, eight died (80% mortality). None of the HCWs died No HCW reported contact with camels or camel products.

Other measures taken by the infection prevention and control (IPC) department included, isolating patients and HCW in RU and cardiac ward with suspected infection till results were negative, HCWs who tested positive for MERS-CoV were isolated at home and were only allowed back to work with two subsequent negative PCR at least 24 hours apart, inpatients who tested MERS-CoV-positive were placed in AIIR new, admissions for elective procedures were postponed. Other measures included increasing space between patient beds to >3 meters in ER, placing a physical ceramic barriers between beds in RU instead of curtains between beds, eliminating “disaster beds”, use of disposable curtains at bed entry points, allocating a new mobile building outside ER for triaging and screening patients with acute respiratory illness (ARI), strict adherence to IPC measures with log-in and log-out checklist for each personal

protective equipment (PPE) item used by HCW, 14-days of sick leave (the incubation period) to all known MERS-CoV negative asymptomatic HCW contacts.

***Phylogenetic analysis*:**

MERS-CoV PCR-positive samples from 21/23 individuals were subjected to NGS (2 samples were of poor quality and could not be analyzed further). The phylogenetic analysis and single nucleotide polymorphisms (SNPs) showed the following patterns:

a. 11 of the 18 genomes clustered phylogenetically supporting a single origin of the infection chain (**Figure 1a, Lineage A1**). Three genomes (from Case #6, Case #12, and Case #23, = Lineage A0) and the Case #2 genome were basal to Lineage A1 (Figure 2A) This can also be seen in the pattern of SNPs across the genome set with all genomes sharing SNPs or derived from earlier genomes by the additional of one or a few SNPs (**Figure 1b**). Of interest, Case #2 differed from Lineage A0 by a single nucleotide (position 3932). Lineage A0 to A1 differed by a single nucleotide (Figure 1b).

b. Within the clusters there were epidemiological features (shared room, contact, or caregiver, with appropriate timing) that supported a transmission chain. For example, the genomes from Case #6, Case #12 and Case #23 clustered closely phylogenetically, and shared unique SNPs (**Figure 1b**). The linked Case #6 and Case #12 shared a room and Case #23 was in the next room. Furthermore Case #4 shared a room with Case #2 providing links to later cases and HCW_5 and HCW_11 cared for Case #4.

c. The genome from Case #2 appears basal to the cluster and this indicates that Case #2 may be the source for the outbreak (rather than Case #1 which was implicated by the clinic-epidemiological outbreak investigation).

d. The genome from HCW #16 has multiple SNPs that are not shared with any other genomes (**Figure 1b**). HCW #16 was mildly symptomatic and had contact with Case #4.

e. Minor variant analysis (Figure 1c) was performed for the 18 samples at 4 genome positions showing changes in the consensus genome across the outbreak (positions 3932, 9365, 9839, 24029). Especially relevant, the sample from HCW #16 showed minor variants at these 4 positions that linked the sample with the Case #2, Case #4, the patient HCW #16 cared for, and the Lineage A1, A0 and B genomes.

Compliance with infection prevention and control measures:

68 HCWs (35 from ER, 12 from ICU, 10 from cardiac ward, 6 other) were randomly assessed for compliance with ICP practices including different PPE components: use of gown 41 (compliance: 62.1%), use of gloves 46 (compliance: 69.7%), use of surgical masks 37 (compliance: 57.6%). Eleven of 68 did not use PPE during patient care. When compliance for the five moments of hand hygiene practice was assessed: 55 (83.3%) were compliant. Among the same group, involvement in high risk practices were: Nasopharyngeal (NP) swabbing; 39 (59.1%); nebulization 21 (31.8%); respiratory suctioning 22 (33.3%); intubation 5 (7.6%); sputum induction 9 (13.6%); and handled viral transport media (VTM) 5 (7.6%). HCW #5, HCW #11, HCW #16, HCW #19 did not use N95 mask during aerosolizing procedure, HCW #22 used non-fit tested N95 mask during intubation.

DISCUSSION

Several studies have described outbreaks of MERS-CoV linked to crowded specialist facilities in hospitals such as emergency departments, renal wards, renal dialyses units, and ICUs (32, 33, 34) or in closed dormitory settings such as hostels (35). Whilst MERS-CoV outbreaks can occur in any inpatient ward, they have not yet been described from other specialist units. Our study is the first to report a nosocomial outbreak of MERS-CoV in a cardiac unit setting.

Several factors that have been attributed to increased risk of nosocomial outbreaks, susceptibility to MERS-CoV infection and transmission during the outbreaks. These include: high viral load in MERS case clinical samples, lack of clinical awareness of the possibility of MERS-CoV infection at first patient presentation; overcrowding in inpatient wards; and poor adherence to infection prevention and control measures, and increased host susceptibility due to co-morbidities (12,15,36,37). All these factors applied to this outbreak at KKUH (Figure 2). There was delayed recognition of index case, and of other MERS-CoV-infected patients and HCWs and poor institution of IPC measures by staff. Clinical samples from several patients had very low cycle threshold (Ct) values (which indicates a high viral load by continuous, semi-quantitative measurements of viral load), and may have contributed to high risk of contamination and spread.

Over the past six years, several nosocomial outbreaks of MERS have been reported from hospitals within KSA and other countries (6-16). Most nosocomial outbreaks have focused on identifying the index case as potential source of the outbreak. This is usually done through an epidemiological investigation which may not be accurate without phylogenetic analyses of MERS-CoV strains causing the outbreak. This is illustrated in our study where the first MERS case to be identified (Case_#1) was erroneously labelled as the as index case by the outbreak epidemiological investigation. The patient had presented to two hospitals with symptoms of ischemic heart disease. A careful retrospective review of his medical records at both hospital facilities and his social history did not identify any possible source of his MERS-CoV infection, either at home or in the hospitals. He only became ill with MERS-CoV as an inpatient at KKUH and was phylogenetically linked with the actual index Case_#2.

An analysis of eleven healthcare-associated MERS-CoV outbreaks in Saudi Arabia and the Republic of Korea between 2015–2017 found twenty-five percent of MERS cases who acquired nosocomial infection were healthcare personnel (12). A previous study of 280 household contacts of 26 index MERS-CoV-infected KSA patients, with follow-up serologic analysis in 44 contacts determined the rate of ‘silent or subclinical’ secondary infection after exposure to primary cases of MERS-CoV infection (6). Twelve probable cases of secondary transmission, and seven apparently healthy household contacts were MERS-CoV positive in their upper respiratory tract. Another study reported low levels of MERS-CoV RNA from asymptomatic subjects from MERS-CoV outbreaks in a Jeddah hospital indicating MERS-CoV carriage after exposure to infected patients (10). In our study 13/23 MERS-CoV infected cases were HCWs. Interestingly, our study

detected one asymptomatic HCW whose samples yielded a MERS-CoV genome that could not be directly linked to any other case in the investigation. Our study found 5 mildly symptomatic HCWs whose samples yielded >75% full MERS-CoV genome sequences. Although the direction of transmission cannot be inferred from viral sequence data and it is possible that they were sources of new MERS-CoV infections. This MERS-CoV 'carrier phenomenon' in HCWs requires further study in greater detail to determine its contribution to the spread of MERS-CoV in inpatients with co-morbidities and other risk factors for acquiring MERS-CoV and succumbing to it. Our study highlights the heterogeneities in the epidemiological profile at the start of healthcare associated outbreaks, and the need to better understand the natural history of asymptomatic infection and role of mildly symptomatic HCWs in MERS-CoV nosocomial transmission.

The impact of MERS-CoV on HCWs, patients and their contacts can be devastating and efforts to be into training and certifying HCWs need to be vigorously pursued and sustained (36-39). Whilst MERS-CoV educational campaigns over the years have heightened awareness, our study shows that compliance with IPC measures can wax and wane. In addition, the strict application of standard IPC with compliance with best practices in wearing appropriate personal protective equipment (PPE) needs to be reinforced in all areas of HCFs especially in the emergency room and in all specialist inpatient wards where there are a large MERS-CoV susceptible co-morbid patients. An additional challenge when it comes to HCWs in KSA, is that they are housed in special shared facilities next to hospitals, something unique to this region, leading to continued exposure of HCWs in their accommodation and magnifying nosocomial outbreaks. At

KKUH, nurses live in two compounds where they share the same amenities, cafeteria and gymnastics hall.

Our study suffers from a time lag between the occurrence of outbreak, conduct of the studies, collation of results and time to submission of publications. Other studies of MERS outbreaks from KSA have also encountered this. A study of risk factors for infection among 19 cases (8 MERS-CoV-PCR+ and 11 serologically positive individuals) identified during a MERS outbreak in an all-female dormitory Riyadh in 2015 where direct contact or sharing a room with a known case occurred was only published recently (35). Whilst educational campaigns are leading to increased awareness of MERS-CoV among HCWS, and the number and size of nosocomial outbreaks appear to have decreased over time (26). The FAO-OIE-WHO MERS Technical Working Group (40) met in 2018 and one of their priorities for MERS-CoV research remains mapping of MERS-CoV infection in humans. Performing clinical, epidemiological, and infection control studies during an outbreak are logistically and operationally difficult and accurate identification of the index case and transmission patterns may not be possible without MERS-CoV genomic information. A more coordinated effort at conducting research during outbreaks is required.

Conclusions:

We report a detailed analysis of a nosocomial outbreak of MERS-CoV within the King Khalid University Hospital in Riyadh, Kingdom of Saudi Arabia. During 2 months in 2015, there were 23 laboratory confirmed MERS cases of which 13 were HCWs. Interestingly, phylogenetic analyses identified an index case as different from that assumed from clinico-epidemiological investigation. This manuscript highlights the need

to use genomic/phylogenetic analyses to identify the index case and possible transmission routes so as to improve infection control.

Declarations Section:

Author contributions: MB and ZAM initiated and coordinated the study. MC coordinated sequencing of the virus samples, assembled viral genomes and organized GenBank submissions. MC and MVTP performed analysis of sequence data. All authors contributed equally to the study, analysis of data and writing of the manuscript. All authors read and approved the final manuscript.

Funding for the study: None. Study was performed as part of the clinical service and infection control response investigations and MERS-CoV surveillance**.**

Competing interests:

All authors have an interest in infectious diseases with epidemic potential. All authors declare no financial or other non-financial competing interests

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article and can be made available upon reasonable request. The MERS-CoV short read data have been deposited in the European Nucleotide Archive with the accession numbers ERR963094 - ERR963114

Ethics Approval and Consent to Participate: The King Khalid Hospital Ethics Review committee approved the study Hospital's Institutional Review Board (IRB) number: E-15-1464 and consent obtained was oral.

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LEGENDS TO TABLE AND FIGURES

LEGEND TO TABLE 1: Epidemiological and Clinical characteristics of confirmed MERS cases. Table 1 depicts by patient or Health Care Workers (HCW) numbered 1 to 23 in sequence of diagnosis, possible source or area where MERS-CoV infection occurred, level of care for patients or type of contact for HCWs, severity of symptoms, type of isolation if in hospital or dormitory or home, number of days from exposure to PCR positivity, sample cycle threshold (Ct) values, management outcome and number of days for MERS-CoV-PCR to become negative.

LEGEND TO FIGURE 1:

Figure 1a:

Bayesian phylogenetic tree of the 18 MERS-CoV genomes from this reported hospital outbreak.

Bayesian phylogenetic tree of the 18 MERS-CoV genomes from this reported hospital outbreak. The genome name was annotated with ‘cs’ for ‘case’ and ‘ct’ for contact (HCW). The taxon node was colored according to their corresponding clinical outcome, symptomatic/recovered (turquoise) or died (red). The Bayesian posterior probabilities of higher than 0.75 were given at each node. The tree was mid-point rooted for clarity. All horizontal branch lengths were drawn to the scale of nucleotide substitutions per site with the scale bar indicated in nt substitutions per site

Figure 1b:

All genomes or partial genome sequence from the outbreak were aligned in MAFFT (please refer to Materials and Methods for more info), based on which the nucleotide differences from the putative index genome from Case 2 (16023_1_cs2_0734) were identified. Nucleotide changes to A were marked in orange, to T in red, to G in dark blue, to C in light blue and gaps in the second genome with marked in grey. The

positions of the major MERS-CoV genes are shown in the upper panel. The major cluster A1, the minor clusters A0 and B and the outlier genome from Case_#1 and HCW#16 are marked to the right of the panel.

Figure 1c

Minor variants in the short read data at the 4 sites of nucleotide polymorphism (positions 3932, 9365, 9839, 24029) were detected as previously described (ref37) using Ack (https://beyondgrep.com/documentation/) to count 21 nt kmers with the centered on each polymorphism. The fractions of each variant nucleotide at each positions are plotted. Lineages and genomes are indicated to the left.

LEGEND TO FIGURE 2:

Epidemiological link of all 23 individuals, Large grey background indicates cases within King Khaled University hospital while smaller grey background indicates Prince Mohamed Hospital (PMAH): HCW: Health care worker, CT: Cycle threshold, ICU: Intensive care unit, ER: emergency room.

Dotted lines indicate possible epidemiological link, solid line indicates confirmed epidemiological link

Table 1: Epidemiological and Clinical characteristics of confirmed MERS cases

No.	MERS Case	Possible source case	Area	Level of care/ type of contact risk	Days to +ve MERS-CoV test after exposure	No. of tests before MERS-CoV positive	Clinical sample Cycle threshold (CT) value	Severity of Symptoms	Place of isolation	Outcome : Death or Days to -ve MERS-CoV test
1	patient	Epidemiologically Suspected Index case	Cardiac Ward	ICU	10	1st	14	Severe	Hospital	Died
2	patient	Phylogenetically confirmed Index case	ER	ICU	7	1st	22	Severe	Hospital	Died
3	patient	1	Cardiac ward	ICU	14	2nd	27	Severe	Hospital	Died
4	patient	2	ER	ICU	9	1st	13	Severe	Hospital	Died
5	HCW	4	ER	NP swab	3	1st	26	Mild	Dormitory	15
6	HCW	2	ER	General ward	11	1st	28	Moderate	hospital	10
7	HCW	4	ER	Routine care	5	1st	31	Mild	Dormitory	14
8	HCW	4	ER	Routine care	5	1st	33	Mild	Dormitory	14
9	HCW	4	ER	Routine care	5	1st	27	Mild	Dormitory	14
10	HCW	4	ER	Routine care	10	1st	32	Mild	Dormitory	14
11	HCW	4	ER	NP swab	6	1st	NA	Mild	Dormitory	15
12	HCW	6	Cardiac ward	ICU	3	1st	NA	Severe	Hospital	Died
13	HCW	2	ER	Routine care	14	1st	22	Mild	home	15
14	HCW	2	ER	Routine care	14	1st	NA	Mild	home	14
15	patient	4	Cardiac ward	ICU	12	1st	19	Severe	hospital	Died
16	HCW	4	ER	Suctioning	6	1st	30	mild	home	15
17	HCW	8	ER	casual	3	2nd	NA	none	dormitory	14
18	patient	6	Cardiac ward	ICU	3	1st	NA	severe	hospital	Died
19	HCW	4	ER	Suctioning	8	2nd	NA	mild	dormitory	14
20	HCW	5	ER	Casual contact	6	2nd	30	asymptomatic	dormitory	16
21	patient	15	Cardiac ward	General ward	1	2nd	30	mild	hospital	26
22	HCW	12	ICU	Intubation	14	2nd	NA	severe	hospital	9
23	patient	6	Cardiac ward	ICU	7	1st	35	severe	hospital	Died

HCW: Health care worker, ER: emergency room, ICU: intensive care unit; N/A not available

Figure 1 a

Bayesian phylogenetic tree of the 18 MERS-CoV genomes from this reported hospital outbreak. The genome name was annotated with ‘cs’ for ‘case’ and ‘ct’ for contact (HCW). The taxon node was colored

according to their corresponding clinical outcome, symptomatic/recovered (turquoise) or died (red). The Bayesian posterior probabilities of higher than 0.75 were given at each node. The tree was mid-point rooted for clarity. All horizontal branch lengths were drawn to the scale of nucleotide substitutions per site with the scale bar indicated in nt substitutions per site

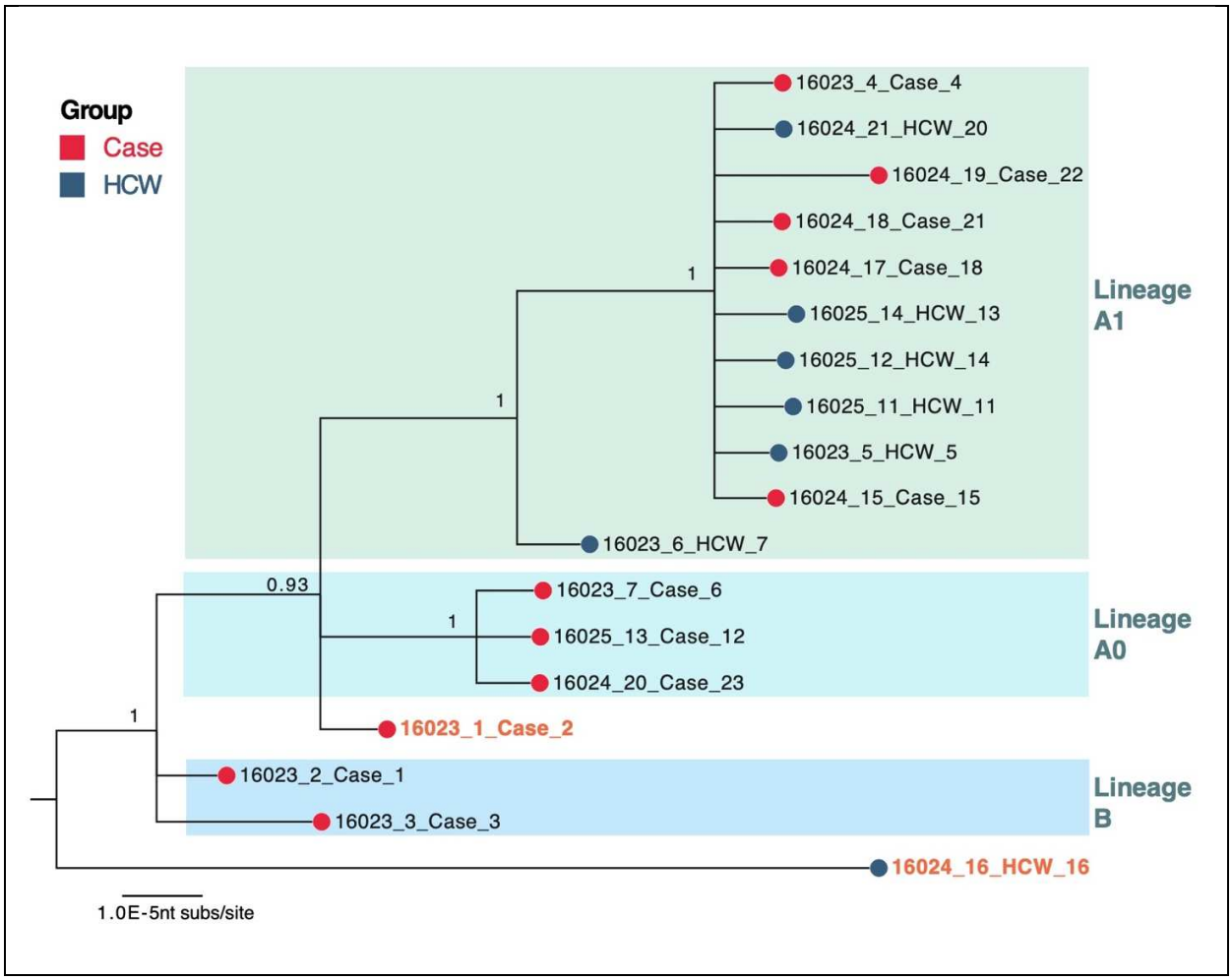

Figure 1b

Genomes sequences from the outbreak were aligned in MAFFT (please refer to Materials and Methods for more info), and nucleotide differences from the putative index genome from Case 2 (16023_1_cs2_0734) were identified. Nucleotide changes to A were marked in orange, to T in red, to G in dark blue, to C in light blue and gaps in the second genome with marked in grey. The positions of the major MERS-CoV genes are shown in the upper panel. The lineages A0, A1 and B are marked to the left of the panel,A. The four polymorphic positions (3932, 9365, 9839 and 24029 and 27482 are indicated.

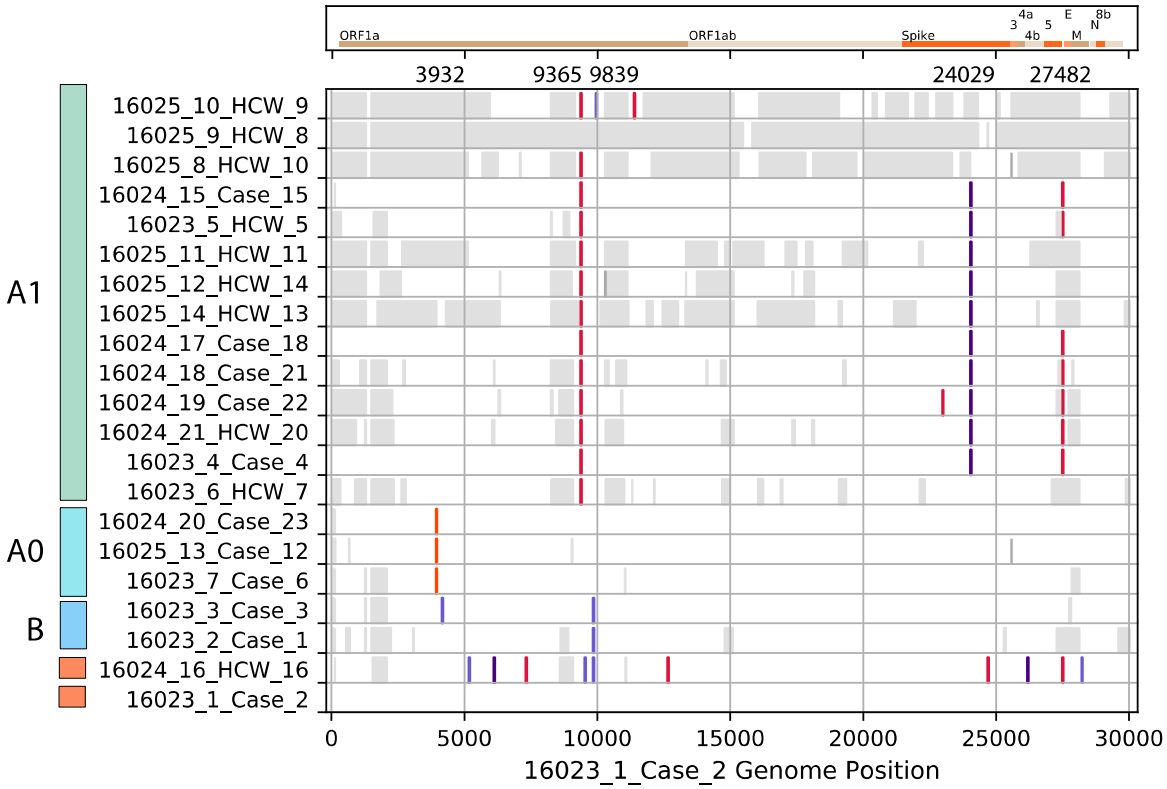

Figure 1c

Minor variants in the short read data at the 4 sites of nucleotide polymorphism (positions 3932, 9365, 9839, 24029, see Figure 1b) were detected as previously described (ref37) using Ack (https://beyondgrep.com/documentation/) to count 21 nt kmers with the centered on each polymorphism. The fractions of each variant nucleotide at each positions are plotted. Lineages and genomes are indicated to the left. Position 27482 also shows variation but coverage was too low for analysis.

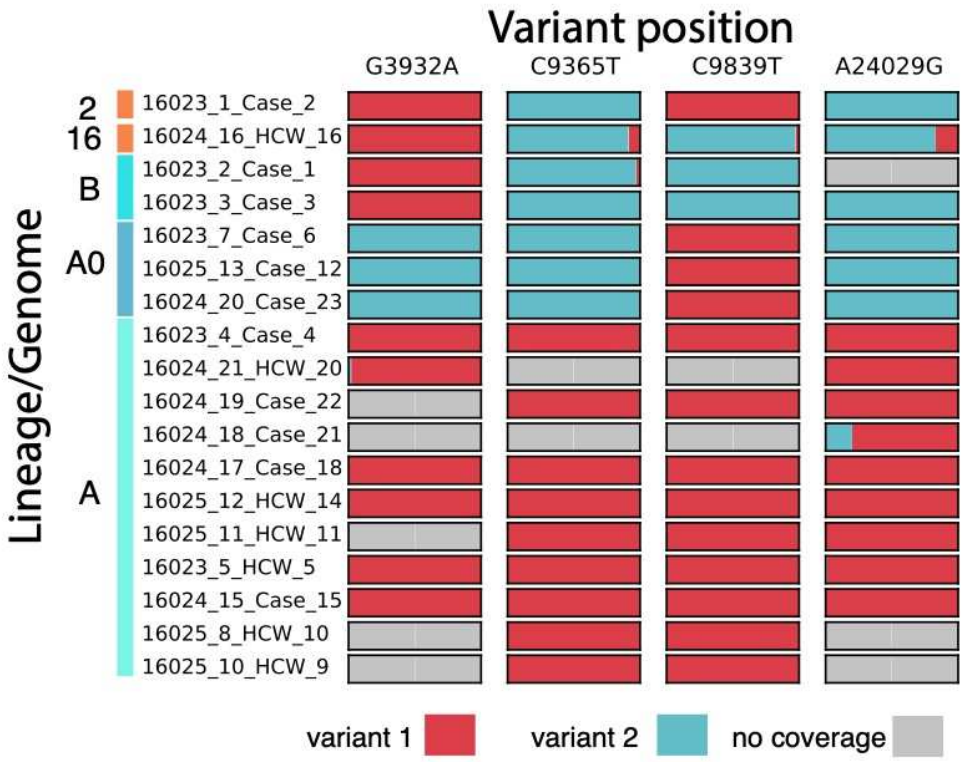
Variant position
G3932A
C9365T
C9839T
A24029G
Lineage/Genome
2
16
B
A0
A
16023_1_Case_2
16024_16_HCW_16
16023_2_Case_1
16023_3_Case_3
16023_7_Case_6
16025_13_Case_12
16024_20_Case_23
16023_4_Case_4
16024_21_HCW_20
16024_19_Case_22
16024_18_Case_21
16024_17_Case_18
16025_12_HCW_14
16025_11_HCW_11
16023_5_HCW_5
16024_15_Case_15
16025_8_HCW_10
16025_10_HCW_9
variant 1
variant 2
no coverage

Figure 2 Epidemiological link of all 23 individuals, Large grey background indicates cases within King Khaled University hospital while smaller grey background indicates Prince Mohamed Hospital (PMAH): HCW: Health care worker, CT: Cycle threshold, ICU: Intensive care unit, ER: emergency room. Dotted lines indicate possible epidemiological link, solid line indicates confirmed epidemiological link