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Scrub typhus in South India: clinical and laboratory manifestations, genetic variability, and outcome

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SUMMARY

Objectives: This study sought to document the clinical and laboratory manifestations, genetic variability, and outcomes of scrub typhus, an often severe infection caused by *Orientia tsutsugamushi*, in South India.
Methods: Patients admitted to a large teaching hospital with IgM ELISA-confirmed scrub typhus were evaluated. Clinical examination with a thorough search for an eschar, laboratory testing, chest X-ray, and outcome were documented and analyzed. Additionally, a 410-bp region of the 56-kDa type-specific antigen gene of *O. tsutsugamushi* was sequenced and compared with isolates from other regions of Asia.
Results: Most of the 154 patients evaluated presented with fever and non-specific symptoms. An eschar was found in 86 (55%) patients. Mild hepatic involvement was seen in most, with other organ involvement including respiratory, cardiovascular, and renal. Multi-organ dysfunction was noted in 59 (38.3%), and the fatality rate was 7.8%. Hypotension requiring vasoactive agents was found to be an independent predictor of mortality ($p < 0.001$). The phylogeny of 26 samples showed 17 (65%) clustering with the Kato-like group and eight (31%) with the Karp-like group.
Conclusions: The presentation of scrub typhus can be variable, often non-specific, but with potentially severe multi-organ dysfunction. Prompt recognition is key to specific treatment and good outcomes. Further study of the circulating strains is essential for the development of a successful vaccine and sensitive point-of-care testing.

1. Introduction

Scrub typhus is a re-emerging zoonotic bacterial infection in the region known as the 'tsutsugamushi triangle' of South and Southeast Asia, the Asian Pacific rim, and Northern Australia.[1–3] The causative organism, *Orientia tsutsugamushi*, is transmitted to humans by the bite of the larval stage (chigger) of the trombiculid mites, most commonly *Leptotrombidium deliense*. *O. tsutsugamushi* is a Gram-negative, obligate intracellular bacterium that infects various cells, including endothelial cells and phagocytes, causing acute vasculitis. Clinical manifestations include an acute onset of fever, headache, myalgia, multiple organ dysfunction, and an eschar at the site of inoculation, which is present in a variable proportion of patients. It can range in severity from a mild, self-limiting disease to, if untreated, a fatal illness in 30–50% of those it affects.[4] Previous reports have loosely classified these varied presentations as: mild disease, respiratory-predominant disease, central nervous system-predominant disease (meningioencephalitis), or sepsis syndrome.[5] The clinical manifestations of cases reported from India show variability when compared to those from other endemic Asian countries.[6] This could be due to differences in the infecting strains, which are known to have a high level of antigenic variation. Antigenic variation is primarily due to differences in the 56-kDa type-specific antigen (TSA).

The 56-kDa TSA is a transmembrane protein, likely involved in adherence to and invasion of target cells. It is unique to the *Orientia* genus, and is one of the major attributes for its exclusion from the *Rickettsia* genus.[7] Within the genus itself, however, the protein is

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highly variable as well as highly antigenic, which has made it the basis for strain differentiation.[8] Initially, serologic testing (via complement fixation), based largely on this protein, was used to identify the original Gilliam, Karp, and Kato 'prototype' strains.[9] However, since then, over 20 unique types have been identified and arranged into 'clusters', initially using further serologic testing and then the genetic sequences of this unique protein. Sequencing of this section of the organism's genome has shown that there are four variable domains (VD I–IV), coding for four hydrophilic regions of this protein.[10,11] Recent efforts to catalog the strains present at various locations have shown that novel subtypes continue to emerge. A recent phylogenetic analysis of *O. tsutsugamushi* from Taiwan reported two unique sequence types along with Karp-related, Saitama, JG-related, and Kuroki-related genotypes.[12] Multi-locus sequence typing (MLST) analysis of Thai isolates has shown a very high rate of recombination, which along with gene duplication and horizontal transfer accounts for the genetic diversity among *O. tsutsugamushi* isolates.[13,14] In 2008, five *O. tsutsugamushi* clades were reported to be circulating in Cambodia and three in Vietnam, with high genetic diversity.[15] In 2011, several isolates, newly characterized from Thailand, were found to be related to Kato and Karp strains.[16] The prevalence of new Karp-like and Kato-like strains is now being reported widely and requires further analysis.[3,17] However, despite this growing knowledge of strain epidemiology and phylogeny in endemic regions, there is a true paucity of information from India.[1] Although there have been a few scattered reports, there is still too little data to create a comprehensive picture of this populous country that is broadly affected by this re-emerging infection.[3,5,18,19] Therefore, in addition to describing the clinical and laboratory manifestations and outcome of the disease, the objectives of this study included describing the genotypes of *O. tsutsugamushi* and comparing these isolates to those from other affected regions.

2. Methods

Patients over 15 years of age admitted with scrub typhus to the Christian Medical College, Vellore, a 2700-bed medical college hospital, between August 2009 and October 2010, were included in the study. Scrub typhus had to be confirmed by IgM ELISA; patients with and without an eschar were included. The study was approved by the Institutional Review Board and Ethics Committee, and informed consent was obtained. A detailed clinical examination, including a careful search for an eschar, was documented for each patient. Basic laboratory studies were performed, including a complete blood count, creatinine, glucose, liver function tests, and chest X-rays; additional investigations were performed when indicated, and included blood cultures, quantitative buffy coat (QBC) testing for malarial parasites, abdominal ultrasound, and serology for leptospirosis and dengue. IgM ELISA was performed on serum samples using Scrub Typhus Detect (InBios International, Inc., Seattle, WA, USA) as per the manufacturer's instructions. An optical density (OD) >0.5 was considered positive.

2.1. PCR analysis

Eschar samples used for PCR were collected and stored in absolute ethanol at −80 °C until use. Bacterial DNA, extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions, was used as the template for the PCR. A standard PCR targeting the 56-kDa protein was carried out on 35 representative eschar samples as reported previously.[3,17] The expected amplicon size was 410 bp. The oligonucleotide primers used were as follows: forward OtsuF: 5′-AATTGCTAGTGCAATGTCTG-3′; reverse OtsuR: 5′-GGCATTATAGTAGGCTGAG-3′.

The PCR amplification mixture (final volume 50 μl) contained 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 200 μM each of dATP, dGTP, dCTP, and dTTP, 0.2 μM of primers, and 1 U of Taq DNA polymerase (Boehringer Mannheim, Germany). The reaction was carried out in a DNA thermal cycler (MJ Research, USA). Amplification consisted of 40 cycles, which included denaturation at 94 °C for 30 s, annealing at 57 °C for 1 min, and extension at 72 °C for 1 min. The PCR products were electrophoresed in 1.5% agarose gels, and the gel containing DNA fragments was stained with 0.5 μg/ml of ethidium bromide and visualized by ultraviolet transillumination (BioRad Inc., USA).

2.2. DNA sequencing

Twenty-six selected amplicons were subsequently purified using the QIAquick PCR Purification Kit (Qiagen) as per the manufacturer's instructions. Sequencing reactions were done using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), followed by enumeration on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were edited using FinchTV (Geospiza Inc.), and phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5.[20] The phylogenetic tree with 1000 bootstrap replicates was constructed using the neighbor-joining method and the distances calculated using the maximum composite likelihood method. The sequences obtained were identified by comparison with sequences available in GenBank using BLAST software (http://blast.ncbi.nlm.nih.gov). Good sequence reads were obtained for 25 eschar samples and the sequences have been submitted to GenBank (accession numbers **KC153061**–**KC153085**).

2.3. Statistical methods

The statistical analysis was performed using SPSS software for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive data are given as the mean ± standard deviation (SD) or as the median and range. The Chi-square test or Fisher's exact test was used to compare dichotomous variables, and the *t*-test or Mann–Whitney test was used for continuous variables, as appropriate. The associations of clinical and laboratory features with the outcome were analyzed by univariate and multivariate logistic regression and 95% confidence intervals (95% CI) were calculated. For all tests, a two-sided *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1. Clinical profile

The study cohort consisted of 154 cases of proven scrub typhus admitted during the study period. The mean age of the patients was 46 ± 15 years, and there was a slight male predominance (81 males, 54%). The majority (77%) were agricultural workers and housewives. Common symptoms included fever, breathlessness, cough, nausea and vomiting, headache, and myalgia (Table 1). The mean duration of fever prior to presentation was 9 ± 3 days. An eschar was present in 86 (55%), with common sites including the groin, axilla, neck, and breast folds.

3.2. Organ dysfunction and outcome (Table 2)

The majority of patients had some form of respiratory complaint on admission, with more than 60% reporting shortness of breath, 37% cough, and tachypnea being observed in nearly three-quarters (71.4%). An abnormal chest radiograph was evident in 61%. Acute respiratory distress syndrome (ARDS) was diagnosed

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Table 1
Patient characteristics[a]

Patient characteristics	Alive, $n = 142$ (92.2%)	Dead, $n = 12$ (7.8%)	p-Value
Age, years	45.14 ± 15.2	50.3 ± 12.8	0.18
Sex, male:female	76:66	5:7	0.5
Duration of illness before admission, days	9.7 ± 3.8	7.3 ± 2.5	0.04
Occupation			
Farmer	68 (47.9)	3 (25)	0.14
Housewife	42 (29.6)	6 (50)	0.19
Other	32 (22.5)	3 (25)	1.00
Co-morbidities			
Diabetes mellitus	21 (14.8)	1 (8.3)	1.00
Hypertension	18 (12.7)	5 (41.7)	0.02
COPD/asthma	5 (3.5)	0 (0)	1.00
Chronic renal failure	1 (0.7)	0 (0)	1.00
Pregnancy	2 (1.4)	1 (8.3)	0.21
Headache	63 (44.4)	3 (25)	0.24
Altered sensorium	34 (23.9)	4 (33.3)	0.47
Seizures	9 (6.3)	1 (8.3)	0.78
Nausea/vomiting	77 (54.2)	6 (50)	1.00
Myalgia	47 (33.1)	3 (25)	0.75
Diarrhea	17 (12)	3 (25)	0.19
Cough	53 (37.3)	4 (33.3)	1.00
Shortness of breath	82 (57.7)	12 (100)	0.004
Rash	2 (1.4)	0 (0)	1.00
Eschar	78 (54.9)	8 (66.7)	0.55
Tachypnea	99 (69.7)	11 (91.7)	0.18
Investigations			
Heart rate/min	102 ± 17	114.8 ± 20.9	0.02
Systolic blood pressure, mmHg	108.8 ± 15.8	106.6 ± 16.6	0.55
Hemoglobin, g/dl	12 ± 1.7	12.5 ± 1.9	0.36
WBC count, $\times 10^9$/l	10.4 (1.2–36.6)	14.4 (7.7–36.0)	0.001
Platelet count, $\times 10^9$/l	79.0 (3.0–368.0)	38.0 (15.0–98.0)	0.03
Bilirubin (mg/dl)	1.1 (0.4–14.6)	2.25 (0.5–8.2)	0.4
Total protein (g/dl)	6.4 ± 0.7	5.8 ± 0.8	0.09
Albumin (g/dl)	2.7 ± 0.5	2.4 ± 0.5	0.07
AST (IU/l)	132 (14–752)	201 (88–325)	0.25
ALT (IU/l)	70 (10–312)	65 (30–117)	0.47
ALP (U/l)	151 (44–715)	200 (83–403)	0.52
Serum creatinine (mg/dl)	1.1 (0.5–6.6)	2.2 (0.6–8.6)	0.001
Creatinine phosphokinase	94 (21–3010)	261 (82–2172)	0.03
Abnormal chest X-ray	83 (58.5)	11 (91.7)	0.03

COPD, chronic obstructive pulmonary disease; WBC, white blood cell count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
[a] Values are given as the mean ± standard deviation or median (range), as appropriate, for continuous variables, and as the number (percentage) for discrete variables.

in 43.5%, and 33.7% required ventilatory support for a mean 4 ± 2 days.

Gastrointestinal and hepatic involvement was common. More than half of the patients (53.9%) complained of nausea and vomiting at the time of admission, with a minority having diarrhea (13.0%). There was also evidence of liver involvement, with a mild transaminitis (elevation to 2–3 times normal) in 72.5% and an elevated bilirubin in 26.6%.

Central nervous system manifestations were not uncommon. Headache was documented in 66 (42.8%) patients, an altered sensorium in 38 (24.6%), and seizures in only 10 (6.5%) patients. Aseptic meningitis or meningoencephalitis was diagnosed in 29 (18.8%) patients.

An elevated creatinine of >2.5 mg/dl was observed in 20 (12.9%) patients, with about a quarter requiring dialysis. Hypotension with a need for vasoactive agents was seen in 38 (24.6%) patients. Over a third of patients (38.3%) had evidence of multi-organ dysfunction. The overall in-hospital mortality was 7.8%, but in the majority of patients who survived (65.5%) there was a dramatic response to doxycycline therapy, with the mean time to defervescence being 2 ± 0.8 days.

3.3. Predictors of mortality

The predictors of mortality were explored using univariate and multivariate analysis (Table 3). The presence of an eschar did not have a statistically significant impact on mortality, nor did pre-existing medical conditions such as diabetes mellitus, hypertension, asthma/chronic obstructive pulmonary disease, or chronic renal failure. On univariate analysis, the factors associated with mortality included the presence of jaundice ($p = 0.02$), hypotension requiring vasoactive agents ($p = 0.001$), ARDS ($p = 0.02$), need for

Table 2
Complications

Complication	No. (%)	Alive (%)	Dead (%)	p-Value	RR (95% CI)
ARDS	67 (43.5)	58 (40.8)	9 (75)	0.03	4.3 (1.1–16.7)
Shock requiring vasoactive agents	38 (24.6)	29 (20.4)	9 (75)	0.001	11.6 (2.9–45.9)
Hepatitis (bilirubin ≥2.5 mg/dl)	99 (64.2)	91 (64.1)	8 (66.7)	0.29	1.9 (0.56–6.3)
Meningoencephalitis	29 (18.8)	29 (20.4)	0 (0)	0.9	-
Renal failure (creatinine ≥2.5 mg/dl)	20 (12.9)	15 (10.6)	5 (41.7)	0.005	6.04 (1.7–21.4)
MODS	59 (38.3)	48 (33.8)	11 (91.7)	0.004	21.5 (2.7–171.7)

CI, confidence interval; ARDS, acute respiratory distress syndrome; MODS, multiple organ dysfunction syndrome; RR, Relative Risk.

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Table 3
Predictors of mortality

	Alive, $n = 142$ (92.2%)	Dead, $n = 12$ (7.8%)	RR (95% CI)	p-Value
Altered sensorium	34 (23.9)	4 (33.3)	1.6 (0.45–5.6)	0.5
Duration of illness, days	9.7 ± 3.8	7.3 ± 2.5	0.82 (0.67–0.99)	0.04
Seizures	9 (6.3)	1 (8.3)	1.3 (0.16–11.6)	0.78
SOB	82 (57.7)	12 (100)	1.14 (1.06–1.2)	0.99
Jaundice	36 (25.7)	7 (58.3)	4.04 (1.2–13.5)	0.02
Presence of eschar	78 (54.9)	8 (66.7)	0.61 (0.17–2.1)	0.43
Heart rate/min	102 ± 17	114.8 ± 20.9	1.04 (1.006–1.07)	0.02
Systolic BP ≤90 mmHg	23 (16.2)	3 (25)	1.7 (0.43–6.8)	0.43
WBC count, $\times 10^9$/l	10.4 (1.2–36.6)	14.4 (7.7–36.0)	0.99 (0.99–0.99)	0.01
Platelet count, $\times 10^9$/l	79.0 (3.0–368.0)	38.0 (15.0–98.0)	0.99 (0.99–0.99)	0.04
Bilirubin (mg/dl)	1.1 (0.4–14.6)	2.25 (0.5–8.2)	1.08 (0.9–1.3)	0.4
Bilirubin ≥2.5 mg/dl	36 (25.4)	5 (41.7)	1.9 (0.57–6.4)	0.29
AST (IU/l)	132 (14–752)	201 (88–325)	1.003 (0.99–1.008)	0.25
ALT (IU/l)	70 (10–312)	65 (30–117)	0.99 (0.98–1.007)	0.47
ALP (U/l)	151 (44–715)	200 (83–403)	1 (0.99–1.006)	0.52
ARDS	58 (40.8)	9 (75)	4.3 (1.1–16.7)	0.03
Hypotension requiring vasoactive agents	29 (20.4)	9 (75)	11.7 (2.9–45.9)	0.001
Serum creatinine (mg/dl)	1.1 (0.5–6.6)	2.2 (0.6–8.6)	1.75 (1.25–2.47)	0.001
Serum creatinine ≥2.5 mg/dl	15 (10.6)	5 (41.7)	6.04 (1.6–13.6)	0.005
Meningoencephalitis	29 (20.4)	0 (0)	0.00 (0.00–0.00)	0.99
Ventilatory support	42 (29.6)	10 (83.3)	11.9 (2.5–56.6)	0.002
Duration of ventilation	0 (0–17)	1 (0–17)	1.06 (0.89–1.25)	0.85
MODS	48 (33.8)	11 (91.7)	0.05 (0.006–0.37)	0.004
Genotype				
Kato-like	16 (66.6)	1 (8.3)	1.4 (0.14–15.4)	0.8
Karp-like	7 (29.1)	1 (8.3)		
Gilliam	1 (4.1)	0 (0)		

RR, relative risk; CI, confidence interval; SOB, shortness of breath; BP, blood pressure; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ARDS, acute respiratory distress syndrome; MODS, multiple organ dysfunction syndrome.
[a] Values are given as the mean ± standard deviation or median (range), as appropriate, for continuous variables, and as the number (percentage) for discrete variables.

mechanical ventilation ($p = 0.004$), and renal failure with a creatinine greater than 2.5 mg/dl ($p = 0.006$). The duration of symptoms and duration of ventilation tended to be higher in non-survivors. Hypotension requiring vasoactive agents was found to be an independent predictor of mortality (relative risk 11.5, 95% CI 2.9–45.15; $p < 0.001$) on multivariate analysis using the forward method, with significant variables of jaundice, renal failure, ARDS, and hypotension requiring vasoactive agents.

3.4. Genotyping data

The 410-bp region of the 56-kDa TSA was amplified from the collected samples (Figure 1), and 26 representative amplicons were sequenced and compared with isolates from other regions of Asia. On phylogenetic and sequence analysis, 17 strains (65%) clustered with the Kato group, eight (35%) clustered with Karp-like isolates, and one with the Gilliam stain (Figure 2). The majority of the isolates showed a 95–98% sequence similarity with *O. tsutsugamushi* isolates from Vietnam and Cambodia. Eighty-one percent of the Kato-like isolates showed 90–95% similarity to the Hualein 3 isolate from Taiwan. The genetic variations of this small number of sequences did not have any significant association with the outcome.

4. Discussion

Scrub typhus affects almost a million people every year and mainly occurs in populations that encounter scrub vegetation as part of their occupation or daily life.[21] The disease, which had been known to be endemic in these regions, was lost from the radar of the scientific community until recently, probably due to the widespread use of pesticides, empiric chloramphenicol and tetracycline use for acute febrile illnesses, and changes in lifestyle. However, the disease is now clearly documented to have

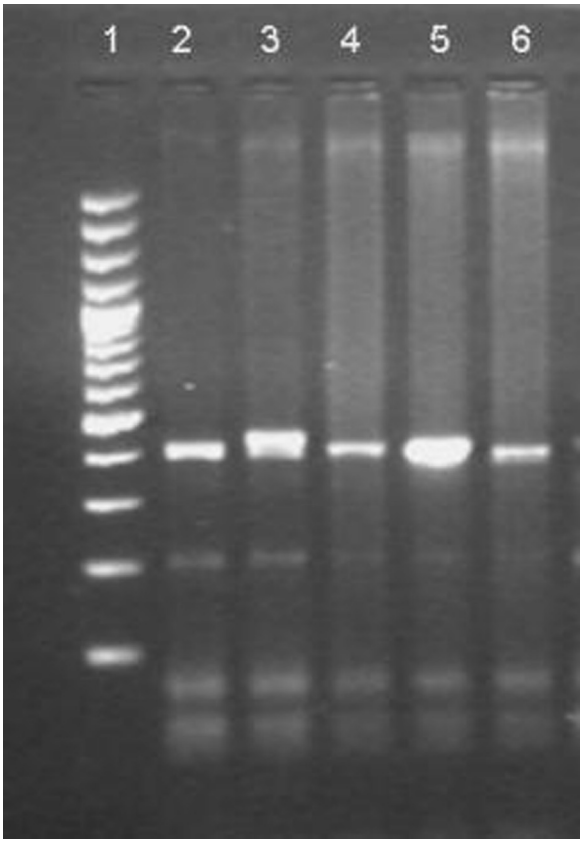

Figure 1. 56-kDa antigen PCR. Lane 1: 100-bp ladder; lane 2: OTV1; lane 3: OTV2; lane 4: OTV4; lane 5: OTV5; lane 6: OTV6. Orientia strains isolated from Vellore are designated as OTV. Non-specific primer binding due to hypervariability in the region could be a possible explanation for the double band seen in lane 3.

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re-emerged on the Indian subcontinent.[22] In this study of 154 cases of scrub typhus admitted to a tertiary care hospital, over a third of patients had multi-organ dysfunction, resulting in a case fatality of 7.8%. Hypotension requiring vasoactive agents was found to be an independent predictor of mortality, and the phylogenetic analysis revealed Kato-like and Karp-like strains to be the predominant circulating ones.

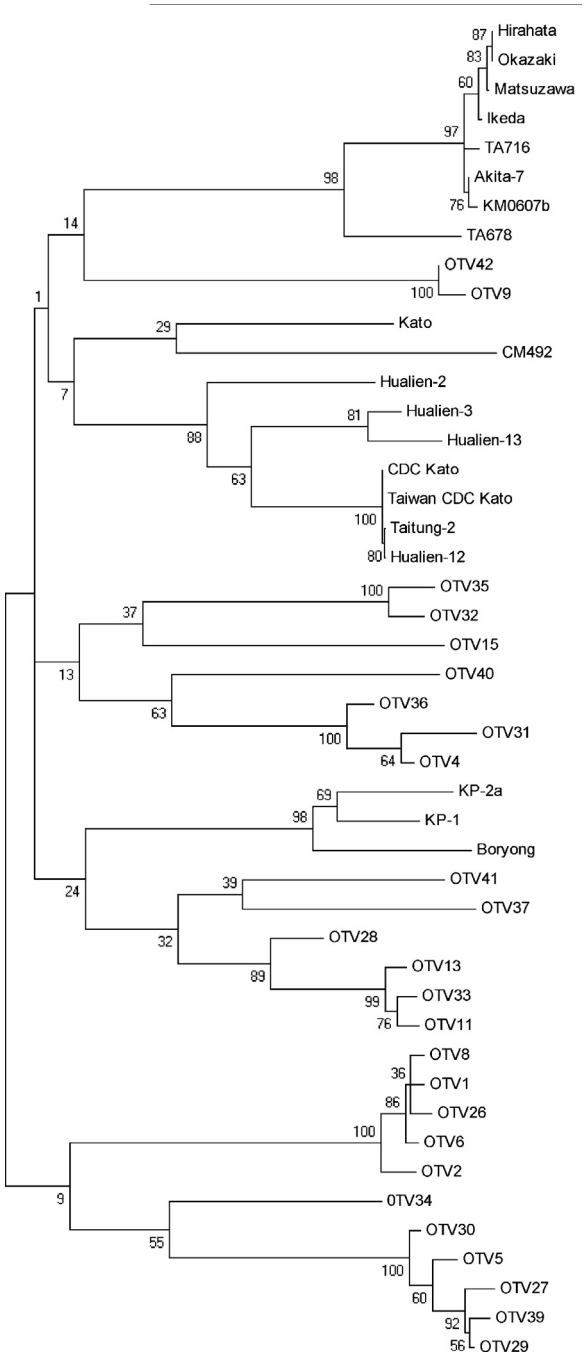

Figure 2. Phylogenetic analysis of isolates using the neighbor-joining method. The evolutionary history was inferred using the neighbor-joining method. The evolutionary distances were computed using the maximum composite likelihood method, and the percentage of replicate trees that clustered together using the bootstrap test are shown next to the branches. The analysis involved 46 nucleotide sequences. Codon positions included are $1^{st} + 2^{nd} + 3^{rd}$ + non-coding. All positions containing gaps and missing data were eliminated. A total of 353 positions were included in the final dataset. Evolutionary analyses were conducted in MEGA5.[20]

4.1. General findings

Scrub typhus has previously been found to account for up to 50% of undifferentiated febrile illness occurring in this part of India during the cooler months of the year,[23] and cases have also been reported from Tamil Nadu, Kerala, Maharashtra, Bihar, Karnataka, Jammu and Kashmir, Uttaranchal, Rajasthan, West Bengal, and Meghalaya.[3,5,24,25] Most of the symptoms of scrub typhus are non-specific in nature, including fever, breathlessness, nausea, headache, vomiting, and myalgia, and do not show much variation among subjects in different studies. However, the presence of the eschar shows considerable variation. An eschar prevalence of up to 90% was reported from Korea.[26] Mahajan et al., however, found the incidence of the eschar in northern India to be far lower at only 9.5%.[3] The incidence of eschar in our study was 55%, which is consistent with prior findings of Vivekanandan et al., who found an incidence of 46% in a hospital near the same geographic locations.[19] Overall, an eschar may be a highly variable finding, found in anywhere from 10% to 92% of patients with scrub typhus, but may not be found, even when present, unless thoroughly searched for.[27] In the present study, the absence of an eschar was not a predictor of mortality, as has been shown in other studies.[26] In fact, though not statistically significant, there was a trend towards a direct correlation between the finding of an eschar and mortality.

Similarly, jaundice on presentation was found to predict mortality. However, liver function tests were not significantly different between those who survived and those who did not. It is unclear what the significance of these findings are, possibly representing a search bias on presentation, with more attention paid, thus more physical findings noted, in sicker patients.

4.2. Complications

In addition to variations in eschar findings, reports of laboratory findings and serious complications vary significantly. Previous studies from India have shown an incidence of ARDS of 8–10%,[3,19] while our data showed 43.5% of patients with evidence of ARDS. This was a predictor of mortality, as shown in previous studies.[5] We found evidence of hepatocellular involvement in 64.2% of our patients, while an earlier study from India has reported an even higher percentage of patients (95.9%) with elevated liver enzymes.[19] The incidence of renal impairment was 13% in this study, which is similar to results obtained by Vivekanandan et al.,[19] but much lower than the 66.4% reported by Mahajan et al.[3] Meningitis and meningoencephalitis were found more often in our study population, with an incidence of 18.8%, compared to prior studies in India, where these were reported at 14% and 9.5%.[3,19] An altered sensorium, however, was not associated with mortality in our study, as has previously been described.[5,26]

4.3. Mortality

In our study, the overall case fatality rate was 7.8%. Rates of fatality have shown considerable variation between studies, though the reasons for this have not been extensively studied. Even within India, these rates have varied considerably, with the previously mentioned cases from Pondicherry having only a 2% case fatality,[19] while most studies have shown somewhat higher rates. Overall there is potentially a trend towards a decreasing mortality from this infection, as reports of a scrub typhus outbreak in northern India in 2004 documented a mortality rate of 17.2%, which subsequently decreased to 14% in 2006.[3,28] This correlates well with the trend in our data, which previously showed a case fatality rate of 14% in 2003.[6]

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Table 4
Prevalence of genotypes at various geographic locations

South India	Taiwan[12,39]	Vietnam[15]	Cambodia[15]	Thailand[32]	Korea[34]	Japan[35]	China[36–38]
Kato-like (65.3%)	Gilliam (50%)	Karp (77%)	Karp (43.5%)	Karp-like (65%)	Boryong (70–80%)	Kuroki (47.5%)	Gilliam (91%)
Karp-like (30.7%)	Kawasaki/Karp (45%)	TA763 (15.5%)	Kato (21.5%)	Gilliam-like (22%)	Kawasaki (25%)	Kawasaki (42.5%)	Karp (9–23%)
Gilliam (3.8%)	Kuroki (14.7%)	JGv[a] (7.5%)	Gilliam (3.5%)	TA716 (4%)	Karp (2–3%)	Karp (10.0%)	Karp mix (72%)

[a] Japanese Gilliam variant.

4.4. Genotyping and epidemiology

The 56-kDa TSA protein is the most abundant protein of Orientia and contains both unique and cross-reacting epitopes. It is responsible for the genetic diversity of *Orientia tsutsugamushi*.[11,29] Diagnostically, using PCR to look for the 56-kDa protein gene in an eschar is an efficient method in the early stages of illness, as confirmed by several studies. A nested PCR to detect the 56-kDa gene has been reported in several studies, but the use of conventional PCR has not been as well investigated.[17,29] Conventional PCR was found to have a good correlation with nested PCR and has been reported to be useful for molecular diagnostics.[17,30]

Using conventional PCR, we found that the isolates in our study clustered in the Karp-like (30.7%) and Kato-like (65%) groups and showed sequence similarity to isolates from Korea, Taiwan, Vietnam, and Cambodia. An increased prevalence of Karp and Karp-like strains has been reported in the Southeast Asian countries, including Malaysia (56%), Thailand (65%), Myanmar (46%), and Philippines (78%).[31–33] Other studies from India have already reported two new genotypes, including one genetically between the Karp and JP-1 types, and another between Saitama and JG types, in addition to Kato, Kawasaki, and Karp type isolates.[3] Boryong is the predominant strain reported from Korea, while studies from Japan and China have shown a mixture of many strains, which differ between the prefectures.[34,35] Several other studies have reported the prevalence of major genotypes circulating in various endemic geographical locations (Table 4).[12,15,32,34–39] Australia and Taiwan have shown a very high incidence of the TA716 strain,[39,40] and seasonal strain variations have been demonstrated in Chinese and Taiwanese studies.[12,41] Information on the geographical spread of this disease would be helpful for clinicians suspecting possible scrub typhus in a patient. Additionally, knowledge of the geographic distribution of the various disease strains will be vital for the production of future diagnostic tests and vaccines. Although there was no significant association between the genotype and the outcome in this study, probably due to the small sample size, future studies looking at this question using a larger sample, powered to detect the difference, would be helpful.

Since this study was conducted in a large university teaching hospital, the inherent referral bias may overestimate the rates of complications. The other limitation is that of the admission diagnosis based on a single serological test. However, up to 10% false-positivity and false-negativity of this test is clinically acceptable in practice.

4.5. Conclusions

Scrub typhus can be a very serious infection that often presents with non-specific symptoms, making it difficult to differentiate from other infections. The laboratory findings and clinical course may also vary significantly, making diagnosis and appropriate treatment difficult. Jaundice, renal failure, ARDS, and hypotension requiring vasoactive agents are predictors of mortality, with hypotension requiring vasoactive agents being independently so. Strain variations may contribute to the variation in presentation, and so further data correlating strain with clinical presentation would be useful for focusing research on appropriate vaccine candidates. Prompt diagnosis and treatment is essential as a delay in treatment can result in significant mortality, and the development of further point-of-care diagnostics could significantly aid this. The prevalent genotypes in our study were those in the Kato and Karp groups. However, given the broad variation seen in the strains of other countries, further prospective epidemiological and molecular studies will be required to identify the prevalent strains and their antigenic variations in this area.

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