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Molecular typing of rickettsia infection in Karnataka-2023

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Abstract
Background: Rickettsial infections are a most common cause of acute febrile illness worldwide. An intensified surveillance and diagnostic analysis lead to know more information of the disease epidemiology, which helps for forethought and enlargement of relevant control/preventive measures against Rickettsial infections.
Materials and Methods: A prospective descriptive study was conducted during the period of January 2023 to December 2023, by screening suspected Rickettsial fever patients attending Private Medical College and Hospital (Bangalore District, Karnataka, India) to brief the immensity of Rickettsial burden, the history of this disease in terms of clinical symptoms and outcome of the infections in the hospitalized Rickettsial patients. The blood samples of suspected patients were tested for Rickettsia genus specific primer and differentiation of scrub typhus group, typhus group and spotted fever group rickettsiae using 47 kDa, gltA and ompB gene using in house real-time PCR with HRM technique. The clinical case definition by World Health Organization was adopted to categorize the Rickettsial cases.
Results: The total enrolment of samples screened in the study was 421, out of which 41 (9.73%) were detected positive for Rickettsial PCR. By rickettsial PCR typing evaluation, spotted fever group was diagnosed in 19 (46.34%) patients, 12(29.26%) had typhus group and 10 (24.39%) patients had scrub typhus group. Though (spotted fever group, typhus group & scrub typhus group) was present in 41 patients, all the patients in this study were recovered well.
Conclusion: In developing countries like India, building of laboratories with advanced capability for diagnosis and combat-mode ready preparedness for the management of Rickettsial cases in emergency situation may reduce Rickettsial -related mortality.

Keywords: Rickettsial fever, scrub typhus group (STG), typhus group (TG), spotted fever group (SFG), RT PCR

Introduction
In India, Rickettsial fever has become a significant public health issue. Rickettsioses are caused by genus *Rickettsia*, scrub typhus infection is due to *Orientia* spp [1] and Q Fever disease was caused by *Coxiella* [2]. The rickettsial diseases discussed earlier are infectious diseases brought on by several intracellular bacterial groups [3]. pathogens were transmitted from several insect vectors to humans by the bite of infected ticks, mites, lice and fleas [4&5]. Diagnoses of rickettsioses are extremely difficult to make, and the disease poses an imminent threat to worldwide public health due to its mortality rate of 9–70% in untreated cases [6-10, 11, 12]. Previously believed to be primarily endemic in the "tsutsugamushi triangle," scrub typhus is now being reported from South America, France, Africa, and the Middle East. This is unmistakably evidence that scrub typhus is spreading beyond of the "tsutsugamushi triangle" [9]. In an analysis of cases in South India, most number of cases are from Karnataka (50%), Andhra Pradesh (32.3%) and Tamil Nadu (17.7%) [13-16]. Only three percent of patients display the characteristic triad of fever, rash, and history of tick exposure when they are first seen within the first three days of illness. On the other hand, a number of conditions that are considered when making a differential diagnosis can also be linked to a rash. These conditions include drug hypersensitivity, idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, immune complex, disseminated gonococcal infection, secondary syphilis, and toxic shock syndrome. In this study, the diagnosis, geographical spread, grouping of Rickettsial disease in Karnataka between January 2023- December 2023 and the need for

constant monitoring of vector born infections are discussed.

Materials and Methods

Microbiological Laboratory is located in Bangalore the state of Karnataka, India. Patients attend private hospitals and PHC from in and around Bangalore as well as from other neighboring districts. Blood samples were received from patients with Rickettsial-like illnesses attending the Pediatric and the Medicine clinics. The patients were diagnosed as having Rickettsial fever based on presentation of standard criteria with fever illness of 2-7 days duration with symptoms like myalgia, rash, headache and arthralgia. The samples were obtained after the sickness underway yet for the majority of the patients, the specific time of collection was not known. Approximately 5 ml blood was received. The samples were examined using a Rickettsia genus-specific primer and a 47 kDa, gltA, and ompB gene applying an in-house realtime PCR with HRM approach to differentiate the scrub typhus group, typhus group, and spotted fever group.

PCR

Extraction of DNA

Blood samples were subjected for DNA extraction using QIA amp Viral DNA Mini Kit (Qiagen). For positive control, equal volume of synthetic plasmid control Typhus Group (TG), Scrub Typhus Group (STG) and spotted fever group (SFG) was used and for negative control-health volunteer blood sample were used. The extracted nucleic acid elution was then stored at –20 °C for later use.

Amplification

Rotor-Gene Q - QIAGEN was used throughout this study for the HRM RT PCR assay. The standard method for RT-PCR and HRM was employed [11]. Initial amplification was performed using the primers against 16s region for pan Rickettsia and those detected positive were subjected for Rickettsial-grouping [12]. The RT-PCR amplification with a total of 25 µl reaction mixture containing 10 µl of DNA, 10 pmol of the 47 kDa, gltA and ompB primers and components of a Type-it HRM PCR Kit (QIAGEN). The amplification involved the following steps: an initial denaturation at temperature of 95 °C for 5 min, followed by 40 cycle repeats of 95 °C for 10 seconds, 54 °C for 15 seconds and 72 °C for 20 seconds with fluorescence monitoring at the 54 °C annealing step on a predetermined FAM Green channel. Melt-curve analysis was performed with increments of 1 °C/30 s (70—93 °C) to determine the peak fluorescence change over time (dF/dT). Positive results are future confirmed by another commercial kit (BioRad, Hercules, CA, USA). [Figure 1]. For every RT-PCR run, a known positive control (positive Rickettsial DNA) and a negative control (Rickettsial negative patients) was included. The size of the RT-PCR products from the amplification of gltA, 47 kDa and ompB genes was 74 bp, 118 bp and 252 bp respectively [13].

Statistical analysis

Implementing the Epi Info version 6.03 program, the data were analysed using the Chi-square test for linear trend and the Chi-square test for proportion. At $P < 0.05$, the results were deemed statistically significant.

Results

During the study period, total number of samples screened 421, out of which 41 (9.74 %) were positive for Rickettsial DNA RT PCR [Table 1]. Of the 421 cases screened (246 males, 175 females), 22 (8.94 %) of the positive were males and 19 (7.72 %) of the positives were females [Table 2]. The overall increased positivity among males during the study period was found to be statistically insignificant ($P = 0.6801$).

Table 1: Month wise distribution of Rickettsia infections.

Month	Male		Female		Total Tested	Total Positive
	Tested	Positive	Tested	Positive		
Jan 23	21	2	21	2	42	4
Feb 23	25	2	17	1	42	3
Mar 23	22	0	8	0	30	0
Apl 23	20	0	6	0	26	0
May 23	14	3	6	2	20	5
Jun 23	23	1	11	1	34	2
Jul 23	17	2	17	2	34	4
Aug 23	11	1	22	2	33	3
Sep 23	21	2	16	2	37	4
Oct 23	22	2	17	2	39	4
Nov 23	27	5	17	2	44	7
Dec 23	23	2	17	3	40	5
Total	246	22	175	19	421	41

Of the 421 cases screened, 223 were less than 12 years and 198 were more than 12 years of age. Among the Rickettsial positives, 8 (25.8%) were pediatric cases and 23 (74.2%) were adults [Table 2]. Samples were collected among the age group from the range of 0-88 years and the mean age was (25 ± 23 years). The overall increase in the Rickettsial positivity among adult group was statistically insignificant with $P = 0.9015$. Among the pediatric age group, positivity was significantly high in 0-1 years age group followed by >50 years age group, with insignificant P value ($P = 0.2206$). Among the 41 positive samples amplified to RT PCR using group specific primers, 17(41.46 %) were positive for Spotted fever group (SFG), 14 (34.15%) were positive for Typhus Group (TG) and 10 (24.39 %) Scrub Typhus Group (STG) positivity was noted in this period of study.

Table 2: Gender wise distribution of Rickettsial infection TG, STG and SFG

Year	Gender	Rickettsial Group	<1	18-35	36-50	>50	Total
2023	Male	Typhus Group (TG)	1	2	1	3	7
		Scrub Typhus Group (STG)	1	2	0	2	5
		Spotted fever group (SFG)	3	2	2	3	10
	Female	Typhus Group (TG)	2	0	1	4	7
		Scrub Typhus Group (STG)	1	1	1	2	5
		Spotted fever group (SFG)	2	2	0	3	7
		Total	10	9	5	17	41

From the grouping of Rickettsial tests it was deduced that 17 cases had SFG, 14 had TFG and 10 were STG. By clinical evaluation, Rickettsial fever was seen in all 41 cases, 13 cases had arthralgia and myalgia. 12 patients had progressed to

having evidence of rash. Though severe rickettsial was present in 41 patients, the mortality rate was nil during the study period due to timely diagnosis and efficient patient care from a clinical perspective. All patients who had rickettsia with complications were admitted and the others were treated as outpatients. This is mainly due to increase in the testing facilities in Karnataka [14].

Discussion

This study was aimed to find out the prevalence of spotted fever, typhus, and scrub typhus and its variation across various geographical areas of Karnataka. In this study, increased no of SFG positivity were observed in all the rickettsial infections. D'Cruz *et al*. in 2017 reported 32.5% rickettsioses positivity in Tamil Nadu [17]. The current study documented 9.74% rickettsioses (STG, SFG & TFG) positivity in Karnataka. The observed positivity of STG and SFG in this study is more than the resulted positivity in Bhutan (STG—22.6%, SFG—15.7%) by Tshokey *et al*. in 2017 [18] and in Northeast India (STG—30.8% and SFG—13.8%) by Khan *et al*. in 2016 [19]. In this study, age was determined to be a significant risk factor for STG, SFG, and TFG; as age developed, elevated the risk for SFG and TFG. The same pattern was noted in Vientiane city [20], Bhutan [18] for STG and for SFG in Gorakhpur [21] and Greece [22] and in Brazil by da Costa *et al*. [23] In this study, it was discovered that a high rate of positive was associated with a greater risk factor for SFG and STG in rural areas. The same was observed for STG in Rajasthan by Bithu *et al*. in 2014 [24], Daniel *et al*. [25] for SF in Greece, Forshey *et al*. in Peru in 2010 [26] and Sri Lanka in 2012 [27] and for MT by Chang *et al*. 2018 in Korea [28] and by Hidalgo *et al*. in Colombia [29]. Weitzel *et al*. SFG were more common in hilly areas with elevation below thousand meters mean sea level like Jawadhi hills and Kalrayan hills. This is supported by our preliminary results published in 2020 [30] and Kularatne *et al*. in 2012 in Sri Lanka [27] who demonstrated that SFG was more prevalent in the hill regions compared to the plains.

In summary, this is the study we have reported rickettsia positives from different geography (Ecology) area of Karnataka state. We used RTPCR for determining scrub typhus, spotted fever and typhus fever. Enhance surveillance in different regions of India are needed & validate the findings of this study. Furthermore, research that focus on vectors will yield important insights into the distinctions between vectors and hosts and how these relate to ecological elements.

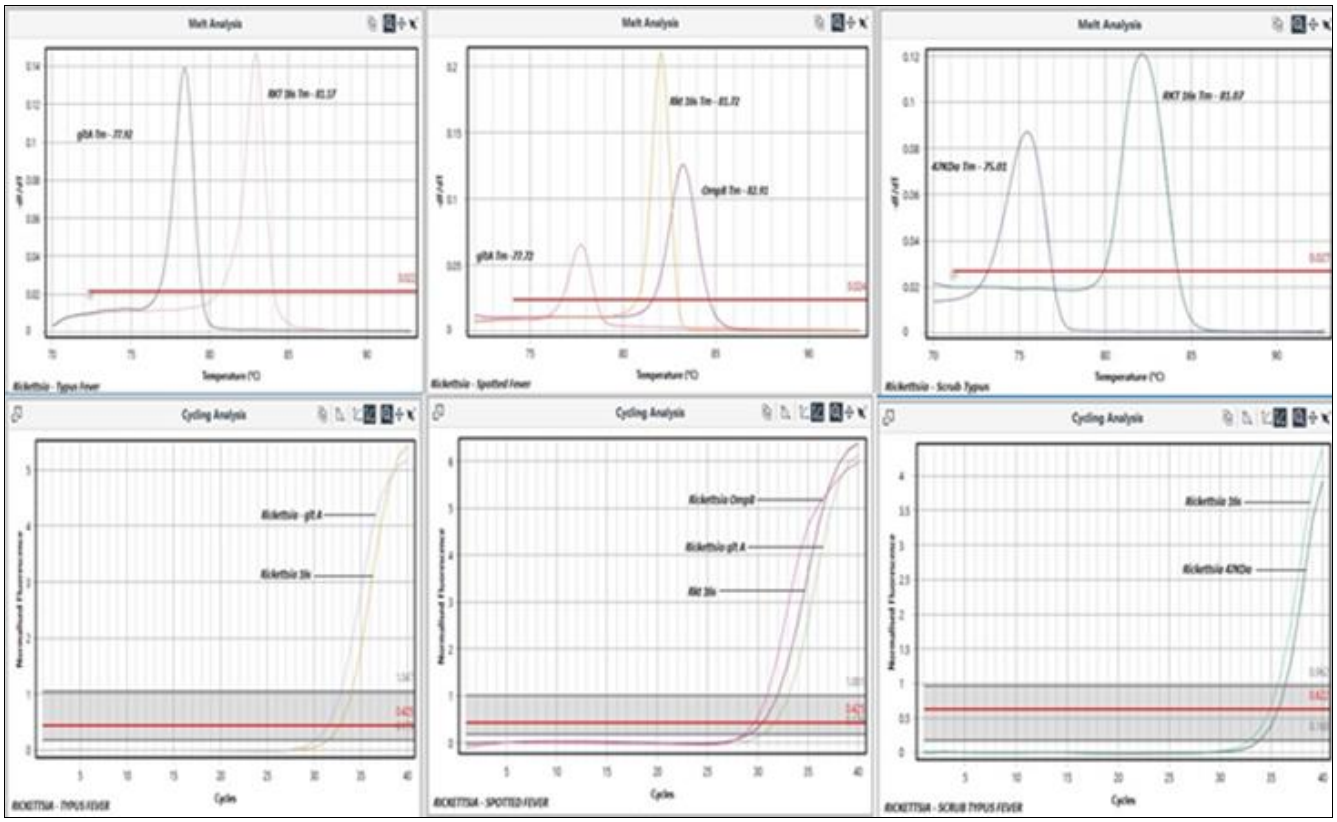

Fig 1: Molecular typing of Rickettsia by RT-PCR with High Resolution Melt Cure Analysis

Conclusion

Rickettsial disease reported in India but poor diagnosis due to the un-aware of the pathogen. Physician and Microbiologists should be aware of the PCR availability for diagnostic tests for the patients. Molecular testing using the rickettsia panel of PCRs is recommended for speciation along with other serological investigations for confirmation.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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