STUDY TITLE

Determination of Etiology of Febrile Illness in Nepal

STUDY ID

DEFINe Study

INVESTIGATORS

Dr. Janak Koirala, MD MPH FACP FIDSA, Professor of Medicine and Infectious diseases (PI)
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Department of Internal Medicine, Patan Academy of Health Sciences
Patan Hospital, Lalitpur, Nepal

GRANT FUNDING

University Grant Commission (UGC) Research Grant (NRS 4,05,900.00)

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STUDY SITE

Proposed Study Site: Patan Academy of Health Sciences, Patan Hospital, Lalitpur

1. ABSTRACT

In the absence of a microbiological lab testing, majority of the cases of febrile illnesses in Nepal are diagnosed on a clinical basis alone. These cases of "undifferentiated febrile illness" are treated empirically with multiple antibiotics without an etiological diagnosis. Unnecessary use of antibiotics can increase the risk of antibiotic resistance and adds unnecessary burden of cost to the patients and their families.

This study aims to determine the etiology of infections using additional microbiological techniques in patients admitted to the hospital with acute undifferentiated febrile illness of 3 days to 21 days duration, and assess its impact on use of antimicrobial drugs. In addition to the clinical features, the etiological diagnosis will be confirmed by using additional tests including culture techniques and ELISA based serological tests for 7 additional pathogens which are currently not tested in Nepal. Patients meeting definition of undifferentiated fever of 3 days to 21 days duration will be enrolled from a major tertiary care center using predefined inclusion and exclusion criteria.

This study will help to identify additional infectious diseases prevalent in Nepal, decrease unnecessary empirical use of multiple antibiotics, and improve patient outcome. Additionally, identification of new causes of febrile illness will help in prevention and control of these infectious diseases at public health level.

2. BACKGROUND AND RESEARCH GAP

Nepal is located between latitudes 26° and 31°N while its altitude ranges from 60m to 8848m from sea level allowing for a large variation in climate. Southern part of Nepal has tropical climate suitable for vector-borne tropical diseases whereas northern parts have more temperate and sub-arctic climate where these diseases are less common. Additionally, water-borne diseases have persisted because of a

lack of sanitation and safe drinking water. Most hospitals in Nepal lack support of well equipped microbiology laboratories for diagnosis of infectious diseases, and hence, most clinicians treat their patients empirically based on their decisions on clinical features alone [1,2]. A study done in the region has shown poor predictability of clinical features alone to distinguish enteric fever from other causes of febrile illness [3].

Either from the lack of or limited access to confirmatory microbiological tests, majority of the cases of febrile illnesses in Nepal are diagnosed on clinical basis only. These cases of "undifferentiated fever" or "undifferentiated febrile illness" are treated empirically without etiological diagnosis. In the absence of correct diagnosis, patients receive multiple empirical antibiotics which increase the risk of antibiotic resistance and adds unnecessary burden of cost to the patients and their families. A hospital-based study of febrile patients published in 2004 revealed identifiable causes in only 37% of the patients [4]. The most common causes of febrile illness in Nepal at the time were enteric fever, murine typhus, scrub typhus, Leptospirosis, and bacterial infections including *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. Studies show a trend in increasing cases of rickettsial infections including *Rickettsia typhi* and *Orientia tsutsugamushi* [4-6].

Preliminary work done at Patan Hospital

Patan Hospital is a tertiary care medical center with 450 beds that admits between 10 to 30 febrile patients per week depending on the season. Over 30% of these patients do not have localizing signs and symptoms for clinical diagnosis nor do they have a laboratory-based diagnosis. These cases are labelled as undifferentiated fever and receive empirical treatment with two or more antimicrobial agents. Previous studies done at Patan Hospital have demonstrated that *Salmonella typhi* and *Salmonella paratyphi A* were causes of undifferentiated fever of >3 days duration in 34% patients and *Rickettsia typhi* in 17% cases, whereas another study in 2008 found 7% cases of febrile illness has *Rickettsia typhi,* which is the cause of Murine typhus [5,6]. These studies show an increase in cases of rickettsial infections including *Riackettsia typhi* and *Orientia tsutsugamushi*. Studies at Patan Hospital have also identified *Streptococcus pneumoniae*, *Leptospira* spp., *Orientia tsutsugamushi*, Hantavirus, and *Coxiella burnetii* as casues of febrile illnesses [4-6].

Research Gap

The epidemiology of infectious disease has changed with massive within country urban migrations, increased migrant workers to India and overseas countries, change in climate, and several other reasons. Previous common causes of febrile illnesses such as malaria and enteric fever have declined over past decade whereas dengue and scrub typhus are on the rise [7-9]. Meanwhile, new diseases such as COVID-19, dengue, and Scrub typhus have become commonplace [5,10,11]. Other causes of febrile illnesses, such as zoonoses (e.g. bartonellosis and Q fever), rickettsial (e.g. Q fever), and viral infections (e.g. arboviruses) remain mostly undiagnosed. This study aims to fill the gap in research by identifying additional causes and estimating burden of these infectious diseases. This will also help to reduce the unnecessary use of antibiotics.

3. SIGNIFICANCE AND INNOVATION

A. Significance

This study is expected to identify additional infectious diseases prevalent in Nepal with the laboratory confirmation of the etiologic agents and improve the appropriate use of antimicrobial agents with targeted therapy. The laboratory tests used in this study will help to determine the presence of additional infectious diseases whose prevalence is currently unknown. This information will be helpful to the clinicians to design initial empiric antibiotic therapy based on epidemiological and clinical features. A more appropriate choice of antibiotics will decrease the unnecessary use of multiple broad-spectrum antibiotics, which in turn will help to reduce antimicrobial resistance, and improve patient outcome. New information discovered from the study will be useful for making policies to prevent and control various infectious diseases such as vector-borne infections and other communicable diseases.

B. Innovation and discovery

The investigators aim to introduce and use selected lab technology with a higher sensitivity and specificity for laboratory confirmation of the diagnosis. We expect to identify new organisms causing febrile illness which are not detected with the currently used routine laboratory tests. Besides confirmation of the diagnosis, this study also plans to compare the clinical diagnosis alone as a guiding tool for the use of empirical antimicrobial therapy versus use of microbiological diagnosis. Hence, we will estimate the overuse and inappropriate use of antibiotics that may lead to antimicrobial resistance. The results and data from this study will be published and shared to educate the clinicians, reduce inappropriate antimicrobial therapy, and improve patient outcome.

4. OBJECTIVES AND SPECIFIC AIMS

A. Overall Objectives and Hypothesis

This study aims to determine the etiology of infections using laboratory methods in patients admitted to the hospitals with acute undifferentiated febrile illness and assess its impact on the use of antimicrobials. In addition to the clinical features of the patients, rapid serological tests and culture techniques will be used to confirm the etiological diagnoses.

We hypothesize that improved determination of etiological diagnoses in patients presenting with undifferentiated febrile illness can decrease the use of broad-spectrum antimicrobials.

B. Specific Aims

Primary:

1. To estimate the proportion of additional infectious etiologies of febrile illness among patients presenting with an undifferentiated fever of 3 to 21 days duration.
2. To compare the use of antimicrobials in patients with undifferentiated fever in whom additional microbiological tests have been performed versus patients with clinical diagnosis alone.

Secondary:

3. To evaluate the appropriateness of the empiric antibiotic therapy based on clinical diagnosis alone and after laboratory confirmation.
4. To estimate the correlation between clinical diagnosis with laboratory diagnosis in patients with undifferentiated fever.
5. To compare the outcome among patients whose treatment is based on microbiological diagnosis compared to the patients whose treatment is based on clinical diagnosis alone.

5. APPROACH: STUDY DESIGN AND METHODOLOGY

This will be a randomized, open-label, prospective study which will include patients admitted to the hospital with an undifferentiated febrile illness of 3-days or longer duration. In addition to the history, physical exam and routine laboratory tests and X-ray, when clinically indicated, a battery of laboratory investigations will be performed to identify the etiology of fever.

a. Definition of Acute Undifferentiated Fever (AUF)

Acute undifferentiated fever (AUF) will be defined as a febrile illness with a temperature of 38°C (100.4°F) or higher for at least a duration of 3 days to 21 days, which presents without an obvious cause on the basis of clinical, radiological, and rapidly available laboratory investigations, and not associated with nosocomial infection, neutropenia, or immunosuppressing conditions [12].

b. Study population
This study will be conducted in the inpatient ward of Patan Hospital.
Patients admitted with undifferentiated fever of at least for 3 days to 21 days duration will be enrolled in this study.

c. Inclusion criteria
   1. Age 16 years or older
   2. Documented fever (T ≥100.4F or ≥38C) of 3 days to 21 days duration
   3. No obvious diagnosis found on the basis of clinical, radiological, or initial routine laboratory tests*
   4. Admitted to the medical unit of hospital
   5. Signed ICF (informed consent form)

   (* Initial routine laboratory tests for an etiologic diagnosis of febrile illness work up include- blood and urine cultures, malaria RDT or thick and thin smears, sputum culture and GeneXpert for MTB (if suspected on chest X-ray), and serologies for scrub typhus, dengue, Dengue, Scrub typhus, Leptospirosis, Brucellosis, Kala azar, and HIV, as indicated based on clinical picture)

d. Exclusion criteria
   1. Fever of less than 3 days or more than 21 days duration
   2. Outpatients

3. Patients being discharged from Emergency Room
4. Children (younger than 16 years in age)**
5. Immunocompromised patients
6. Neutropenia (ANC <500 per cumm)
7. Known case of HIV infection
8. Admission to a hospital for 48-hours or longer within past 30 days

(**Children under 16 years will be excluded since they have different causes of febrile illnesses requiring a different set of lab tests which is beyond the scope of this study.)

e. Informed Consent

Patients admitted to the Medical Ward with febrile illness will be screened for the study inclusion and exclusion criteria. Patients who meet all inclusion criteria and none of the exclusion criteria will be enrolled in the study after taking written voluntary consent on an Informed Consent Form (ICF) approved by the institutional research committee (IRC). If patient is unable to give consent, his or her legal guardian will be contacted for written consent on behalf of the patient.

f. Clinical and Laboratory Procedures

The main purpose of the current study is to find additional causes of febrile illnesses in Nepal which are not tested or detected with the routine laboratory tests, and are generally labelled as an undifferentiated fever. In a patient who meets above inclusion and exclusion criteria and has undergone standard of care evaluation as listed below, the following baseline data including results of the standard of care evaluations will be collected. Then, additional lab tests as noted below will be performed to find an etiology of the febrile illness. These new tests are neither readily available nor routinely performed as a part of the fever work up in Nepal.

Standard of Care Evaluation

Standard of care evaluation of a febrile patient is done by the treating medical team which usually consists of the following set of clinical, basic laboratory, radiological and microbiological evaluations:

1. History and Physical Examination
2. Basic Laboratory investigations: As a part of standard of care, following routine laboratory tests are done on patients with a febrile illness:
   Complete blood count (CBC), Renal function test (RFT), Liver function test (LFT), CRP, ESR, Urine routine exam
3. Radiological investigations: Chest X-ray, USG abdomen, and other Radiological investigations (as indicated)
4. Microbiological investigations:
   a. Blood cultures x 2
   b. Urine culture/sensitivity in symptomatic patients or in patients with >10 pus cells in urinalysis
   c. Culture of sputum, pus, and other samples as indicated based on clinical findings

d. Routine serologies- currently the following serological tests are routinely done for evaluation of fever:

HIV, Dengue, Scrub typhus, Leptospirosis, Brucellosis, Kala azar, malaria (RDT/thick and thin smears)

Additional Evaluations for the Study

For patients without an obvious cause of fever and an inconclusive work up after standard of care evaluations, additional serological tests as a part of undifferentiated febrile illness work up will be performed, including:

a. Clinical evaluation: includes thorough history and physical examinations based on a standard questionnaire and data collection tool
b. Additional Microbiological Evaluations: If above listed laboratory and microbiological tests do not indicate a conclusive diagnosis, the patient's blood will be processed for additional Serological tests as a part of undifferentiated febrile illness work up. These tests will help to establish diagnosis of the following infections:
    - Murine typhus
    - Bartonellosis
    - Q-fever
    - Chikungunya
    - Zika
    - Japanese B encephalitis
    - West Nile virus

5. Use of antimicrobials in the patients with and without an etiological diagnosis and their outcome at the time of discharge from the hospital will be recorded.

g. Laboratory Methods

For the purpose of the study, following samples will be collected from 50% of randomly assigned patients for additional microbiological testing.

1. Blood cultures, 2 sets from 2 different sites from all study patients (20 mL total)
2. Serum samples for serological testing (4 mL)

After obtaining appropriate consent, total 25 mL blood will be collected from each patient assigned to the additional testing arm. Blood samples will be cultured in liquid media and held in the incubator for 7-days.

Urine will be taken to the lab for routine examination and culture will be performed only if there are >10 pus cells or patient reports urinary symptoms.

Gram stain, AFB stain and Genexpert will be performed on sputum samples.

As a part of routine febrile illness work up, the serum samples will be tested for HIV, scrub typhus, dengue, leptospirosis, brucella, malaria, and kala azar.

Additional serum sample will be processed for further serological testing for Chikungunya, Zika, *Coxiella burnetii* (Q-fever), *Bartonella henselae* (Bartonellosis), *Rickettsia typhi* (murine typhus), Japanese B encephalitis, and West Nile virus using ELISA and/or Rapid Diagnostic Test (RDT), as applicable. Although molecular tests have a higher sensitivity and specificity, molecular testing has not been planned for this study because of the budget limits.

h. Data collection

After taking written informed consent from patient or his/her legal guardian, data will be collected from patients who meet all of the inclusion criteria and none of the exclusion criteria. Standard questionnaires will be used and recorded on a designated laptop device protected and secured with password. Only the study data collection and management team will have access to the collected data. Physical exams and initial clinical diagnosis will be transferred from patient's chart, or by interviewing patients, if needed, using a standardized questionnaire. All available lab data and treatment (antimicrobials and other relevant therapy) will be recorded. Chest X-ray, USG, CT scan, and echocardiogram reports will be collected, if applicable to the diagnosis.

i. Follow up and Expected Outcomes

During the hospital stay, patients will be followed up for new laboratory results, changes in treatment plan and outcomes. The patient outcomes will be recorded as:

a. time to defervescence;
b. de-escalation or readjustment in antimicrobials;
c. complete recovery and discharge vs. partial recovery and discharge vs. death.

j. Data Collection, Data Analysis and Sample Size

Demographic, clinical, radiological, and laboratory data will be collected and entered into a password protected database. To minimize the errors, data will be directly entered into the database using REDCap Database questionnaires.

Data analysis will include the followings:

1. Demographics and baseline characteristics of patients will be analyzed and compared between the two groups.
2. Clinical diagnosis and laboratory confirmation of diagnosis will be used to estimate the proportions of various infectious etiologies of febrile illness in patients presenting with a febrile illness of 3-21 days duration. (Specific aim-1)
3. Number, type, dose and duration of antimicrobials used to treat the patients will be collected and compared between the two groups: clinical diagnosis alone versus clinical plus microbiological diagnosis. (Specific aim 2)
4. Appropriateness of the empiric antibiotic therapy will be determined by an independent physician expert after the laboratory confirmation of the diagnosis. Comparison between the two groups (clinical diagnosis vs. microbiological diagnosis) will be done by calculating the proportion of cases who received appropriate antibiotics divided by the total number of cases for whom empiric antibiotics were administered. (Specific aim-3)

5. Pretest probability for clinical diagnosis and posttest probability after laboratory-based diagnosis will be calculated and correlated for bacterial, rickettsial, viral infections versus other causes of fever. (Specific aim-4)
6. Clinical outcomes will be compared for patients whose treatment is based on clinical diagnosis alone (Group 1) versus the patients whose treatment is based on clinical plus microbiological diagnosis (Group 2). Composite Positive outcome (complete or partial recovery) and Negative outcome (in-hospital death) will be compared between the two groups. (Specific aim-5)

Demographics and baseline characteristics of patients will be expressed in percentages, means±SD, 95% confidence intervals, and/or median±IQR, as appropriate. Proportions will be compared between the two groups with Chi-square tests. Numerical variables will be expressed as Means/SD/95% confidence intervals and Median/IQRs, whereas Student T test or Mann-Whitney test, respectively, will be used for statistical comparisons. Alpha will be set at 95% (level of significance, $p < 0.05$).

For Specific aims 2 and 3, total daily defined dose (DDD) for each antimicrobial agent received by patients will be calculated following WHO guidelines. The total DDD, number of antibiotics, and Duration of antibiotics used will be compared between the groups by calculating Means/SD/95% confidence intervals. Similarly, appropriateness of antibiotic uses will be tested with cross-tabulation using Chi-square analysis.

For specific aim 4, pre-test probability and post-test probability will be determined by both using standard calculations and using Fagan's Nomogram. Correlations between pre- and post-test probabilities for bacterial, rickettsial, viral vs. other causes will be evaluated.

For specific aim 5, the hazard ratio using the Cox proportional hazard model will be calculated to compare the outcomes between the groups. Since multiple factors, such as comorbidities, severity of illness at baseline, type of infection and host immune status, can influence the outcomes, adjustment for these factors will be considered during the univariate and multivariate outcome analyses. Updated Charlson comorbidity index (Quant et al.) will be calculated and used for comparison between the groups [15]. Similarly, qSOFA will be used to stratify the patients by severity of infection [16].

REDCap (Research Electronic Data Capture) software will be used for questionnaire and database entry. SPSS version 25 (IBM, USA) will be used for statistical analysis.

Sample Size:

For this study, a total of 234 randomly selected patients with acute undifferentiated febrile illness will be enrolled with a fever of 3 days duration. Of these 234 patients, 117 will be randomly assigned to undergo additional microbiological testing.

Based on previous studies and number of patients needed to include less common seasonal infections, this study will enroll patients throughout a period of 12 months. Cluster sample technique will be used to select 20 patients per month including 10 patients who will be randomly selected to undergo additional microbiological testing.

Sample size determination and assumptions:

For specific aim 1, a reduction of antimicrobial use in about 15% patients is expected after additional laboratory confirmation of the diagnosis including viral infections. Assuming this decrease in antibiotics from 90% to 75%, 95% confidence interval, and 80% power, required sample size would be 97 in each arm. With similar assumptions for specific aim 3, an improvement in antimicrobial therapy from 70% to 90% would require 59 patients.

For specific aim 2, assuming an improvement in identification of causative agents from 30% to 50%, 95% confidence interval, and 80% power, required sample size would be 91.

For specific aim 5, an improvement in clinical outcome for patients whose treatment is based on microbiological diagnosis by 20%, from 60% to 80%, 95% confidence interval, and 80% power, the required sample size would be 79 in each arm.

Based on above calculations, minimum sample size needed would be 97 in each arm. Including a 20% loss for failure to consent and loss to follow up, total sample size needed will be 117 in each arm.

k. Limitations and Alternative Strategies

This study has been proposed for only one center but depending on the findings from this study, it will be expanded to other centers in the future. Additional microbiological methods will be limited to serological testing alone, mainly due to budgets limits. Although molecular tests are more sensitive and specific, molecular testing has not been planned for this study because of the budget limits.

This one center study will serve as a pilot study with limited number of patients has been planned because of the budget limit. However, the results of this study will be useful for treatment of febrile patients. Additionally, the results of this study will be useful for designing larger, multi-centered studies in the future.

6. PROTECTION OF HUMAN SUBJECTS (ETHICAL CONSIDERATIONS)

This clinical research project will be submitted for a full board approval by the PHAS Institutional Research Committee. Patients will be enrolled from Patan Hospital's inpatient ward after taking informed consent on an informed consent form (ICF) as approved by the PAHS IRC. All patients will be provided written information about the study in Nepali language before enrollment. For patients who

are unable to understand Nepali, either a family member or a hospital staff will be used as an interpreter to overcome the language barrier and to help the patient understand the study process and its potential harms and benefits.

This project will have equal opportunities for inclusion of women and minorities. Children will be excluded because of a different spectrum of infectious agents outside the scope of this study.

All personal data collected for the study will be stored in a confidential manner in a secure computer with password protection. Only the research team and assigned data analyst will have access to the database. Any new information received during the study will be revealed to the patient. Patients enrolled in the study will be able to withdraw their consent at anytime they wish. Any serious adverse events will be reported to the IRC.

Registration: After IRC approval, this study will be registered at Clinical Trial.gov.

7. DATA SAFETY AND MONITORING PLAN

All data entered into the database will be secured and password protected. The study principal investigator and co-investigators will review data from time to time for consistency in data collection. An interim data analysis will be done after enrollment of 25% and 50% of the patients.

Data safety and monitoring will be done by an independent committee known as DSM board consisting of two senior level medical doctors including one internal medicine physician and one microbiologist, and a representative of ethics committee or IRC.

The DSMB will review the interim data and events reports to conclude with their recommendations to whether the study should continue without change, be modified, or be terminated.

8. EXPECTED OUTPUTS (STUDENT TRAINING AND PUBLICATIONS)

This study will help the internal medicine residents and infectious disease fellows at PAHS to learn to design and conduct clinical studies. They will also participate in patient enrollment, data collection, analysis and presentation/publication phases of the study.

The results of this study will be shared within and outside the institution in the form of scientific presentations and publication in peer-reviewed journals.

9. ORGANIZATION OF THE STUDY

Study staff will consist of the followings:

Staff category	Number	Role in study
Principal Investigator	1	Design, Supervision, Conduction, Data monitoring, Analysis, Write up for

		publication, and Scientific presentations
Co-Investigators	2	Design, Enrollment, Data collection supervision, Patient monitoring, Scientific presentation and publication
Researchers (Infectious Diseases fellows and Internal medicine residents)	2-3	Patient enrollment, consent, clinical follow up and data collection, data entry
Statistician	1	Data analysis

10. STUDY TIMELINE

Events	Details	Timeline
Preparatory Phase	IRC approval	Jan 2025
	Team preparation	Oct-Dec 2024
	Lab setup/supplies	Oct-Dec 2024
Interventional Phase	Enrollment (12 months)	1 Feb 2025- 31 Jan 2026
	Laboratory tests	1 Feb 2025- 31 Jan 2026
	Follow up	1 Feb 2025- 31 Jan 2026
	Data Collection	1 Feb 2025- 31 Jan 2026
Data Analysis and Reporting Phase	Statistical analysis	1 Feb 2026- 31 Mar 2026
	Report and Manuscript preparations	Apr-Jun 2026

11. DETAILED BUDGET

Budget Item	Unit Cost (Rs.)	Number	Total Cost (Rs.)
A. Personnel			
Dr. Janak Koirala (10% effort)	129011.	0.10	12,900
Dr. Nora Ranjitkar (20% effort)	82074.	0.20	16,414.
Dr. Suman Thapa (10% effort)	76867.	0.10	7686.
B. **Equipment, Consumables, Services**			
Serological test by ELISA and/or RDT	500	7x97	339,500
Other lab supplies for sample collection, processing and transportation of samples	200	97	19,400
C. Travel/Transportation of sample	NA	NA	NA
D. Facilities and Administrative			
Presentation and publication	10,000	1	10,000
TOTAL =			**Rs. 405,900.**

12. ASSOCIATION TO NATIONAL PRIORITY

Under the Epidemic and Outbreak Surveillance Programme of Government of Nepal, Ministry of Health and Population (MOHP), the Early Warning and Reporting System (EWARS) has been set up in 81 sites of the country. However, the laboratory testing for various new infectious diseases prevalent in the Southeast region are not available at these sites or in major hospitals of Nepal. This study is expected to identify additional infectious diseases prevalent in Nepal with the laboratory confirmation of the etiologic agents.

Increasing antimicrobial resistance has been identified one of the major causes of morbidity and mortality in hospitals. Therefore, control of antimicrobial resistance has been another national priority. and improve the appropriate use of antimicrobial agents with targeted therapy. The laboratory tests used in this study will help to identify additional infectious diseases. This information will be helpful to the clinicians to design initial empiric antibiotic therapy based on epidemiological and clinical features. A more appropriate choice of antibiotics will decrease the unnecessary use of multiple broad-spectrum antibiotics, which in turn will help to reduce antimicrobial resistance, and improve patient outcome. New information discovered from the study expected to be useful for making policies to prevent and control various infectious diseases such as vector-borne infections and other communicable diseases.

13. BIBLIOGRAPHY AND REFERENCES

1. Pokharel S, Karki M, Acharya B, Marasini B, Arjyal A. Outbreak of acute undifferentiated febrile illness in Kathmandu, Nepal: clinical and epidemiological investigation. BMC Infect Dis. 2020 Jan 30;20(1):89. PMID: 32000695.
2. Koirala KD, Chappuis F, Verdonck K, Rijal S, Boelaert M. Persistent febrile illnesses in Nepal: A systematic review. Indian J Med Res. 2018 Oct;148(4):385-395. PMID: 30666001.
3. Aiemjoy K, Tamrakar D, Saha S, Naga SR, Yu AT, Longley A, Date K, Hemlock C, Qamar FN, Saha SK, Luby SP, Garrett DO, Andrews JR, Bogoch II. Diagnostic Value of Clinical Features to Distinguish Enteric Fever From Other Febrile Illnesses in Bangladesh, Nepal, and Pakistan. Clin Infect Dis. 2020 Dec 1;71(Suppl 3):S257-S265. PMID: 33258936.
4. Murdoch DR, et al. The etiology of febrile illness in adults presenting to Patan Hospital in Kathmandu, Nepal. Am. J. Trop. Med. Hyg., 70(6), 2004.
5. Thompson CN, Blacksell SD, Paris DH, Arjyal A, Karkey A, Dongol S, Giri A, Dolecek C, Day N, Baker S, Thwaites G, Farrar J, Basnyat B. Undifferentiated febrile illness in Kathmandu, Nepal. Am J Trop Med Hyg. 2015 Apr;92(4):875-878.
6. Zimmerman MD, Murdoch DR, Rozmajzl PJ, Basnyat B, Woods CW, Richards AL, Belbase RH, Hammer DA, Anderson TP, Reller LB. Murine typhus and febrile illness, Nepal. Emerg Infect Dis. 2008 Oct;14(10):1656-9. PMID: 18826840.
7. Rijal KR, Adhikari B, Adhikari N, Dumre SP, Banjara MS, Shrestha UT, Banjara MR, Singh N, Ortegea L, Lal BK, Thakur GD, Ghimire P. Micro-stratification of malaria risk in Nepal: implications for malaria control and elimination. Trop Med Health. 2019 Mar 27;47:21. PMID: 30976192.

8. Poudyal P, Sharma K, Dumre SP, Bastola A, Chalise BS, Shrestha B, Poudel A, Giri A, Bhandari P, Shah Y, Poudel RC, Khadka D, Maharjan J, Ngwe Tun MM, Morita K, Pandey BD, Pandey K. Molecular study of 2019 dengue fever outbreaks in Nepal. Trans R Soc Trop Med Hyg. 2021 Jun 2;115(6):619-626. PMID: 32987406.
9. Gupta BP, Haselbeck A, Kim JH, Marks F, Saluja T. The Dengue virus in Nepal: gaps in diagnosis and surveillance. Ann Clin Microbiol Antimicrob. 2018 Jul 16;17(1):32. PMID: 30008269.
10. Basnet S, Koirala S, Pandey B, Koirala J. COVID-19 Containment Efforts of a Low-Resource Nation: The First Four Months in Nepal. Cureus. 2020 Jul 1;12(7):e8946. PMID: 32765991.
11. Lamichhane P, Pokhrel KM, Alghalyini B, Zaidi ARZ, Alshehery MZ, Khanal K, Bhattarai M, Yadav A. Epidemiology, clinical characteristics, diagnosis, and complications of scrub typhus infection in Nepal: a systematic review. Ann Med Surg (Lond). 2023 Sep 5;85(10):5022-5030. PMID: 37811079.
12. Susilawati TN, McBride WJ. Undiagnosed undifferentiated fever in Far North Queensland, Australia: a retrospective study. Int J Infect Dis. 2014 Oct;27:59-64. Epub 2014 Aug 27. PMID: 25173425.
13. Epidemic and Outbreak Surveillance Programme. Government of Nepal, Ministry of Health and Population, Epidemiology and Disease Control Division. Available at: https://mohp.gov.np/program/epidemic-and-outbreak-surveillance-programme/en (Accessed on 8 Dec 2023)
14. National Action Plan For Antimicrobial Resistance Nepal. Government of Nepal, Ministry of Health and Population, 2021-2026.
15. Quan, H. *et al.* Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am. J. Epidemiol. **173**, 676–682.
16. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3) JAMA. 2016;315(8):801–810.

APPENDIX – 1.

DEFINe Study Questionnaire for Database

Screening Questions

A. Definition of Acute Undifferentiated Fever (AUF)

Acute undifferentiated fever (AUF) will be defined as a febrile illness with a temperature of 38°C (100.4°F) or higher for at least a duration of 3 days to 21 days, which presents without an obvious cause on the basis of clinical, radiological, and rapidly available laboratory investigations, and not associated with nosocomial infection, neutropenia, or immunosuppressing conditions [10].

No.	Question	Yes	No
a1	Temperature 38C (100.4F) or higher?		
a2	Duration of fever > 3 days and <21 days?		
a3	Cause of fever unknown after routine work up including clinical, radiology and lab evaluations?		
a4	Is it a Nosocomial infection?		
a5	Neutropenia ruled out?		
a6	Immunosuppression present?		
a7	**Does the patient meet all criteria for undifferentiated fever? (Needs to answer "Yes" to 1,2&3, and "No" to 4,5,&6)**		

If answer to a7 is "Yes", proceed with questions in "b. Inclusion/Exclusion criteria"

B. Inclusion/Exclusion criteria

Patients needs to meet al of the following Inclusion Criteria before enrollment:

No.	Question	Yes	No
b1	Age 16 years or older		
b2	Documented fever (T ≥100.4F or ≥38C) of 3 days to 21 days duration		
b3	No obvious diagnosis found on the basis of clinical, radiological, or initial routine laboratory tests*		
b4	Admitted to the medical unit of Patan Hospital		
b5	Informed consent form (ICF) signed		
B6	**Patient meets all of the above inclusion criteria**		

(* Initial routine laboratory tests for an etiologic diagnosis of febrile illness work up include- blood and urine cultures, malaria RDT or thick and thin smears, sputum culture and GeneXpert for MTB (if suspected on chest X-ray), and serologies for scrub typhus, dengue, Dengue, Scrub typhus, Leptospirosis, Brucellosis, Kala azar, and HIV, as indicated based on clinical picture)

Patient should meet none of the Exclusion criteria below:

No.	Question	Yes	No
b7	Children (younger than 16 years in age)**		
b8	Fever of less than 3 days or more than 21 days duration		
b9	Outpatients		
b10	Patients being discharged from Emergency Room		
b11	Immunocompromised patients		
b12	Neutropenia (ANC <500 per cumm)		
b13	Known case of HIV infection		
b14	Hospital admission for 48-hours or longer within past 30 days		
b15	**Patient does not meet any of the above exclusion criteria**		

(**Children under 16 years will be excluded since they have different causes of febrile illnesses requiring a different set of lab tests which is beyond the scope of this study.)

If patient meets all of Inclusion criteria and none of the exclusions, proceed with patient consent followed by randomization and enrollment.

Randomization Result

	Information	Remarks
Patient's study ID no.	D001, D002, D003,……..	
Date of Birth (in AD)	DD/MM/YYYY	
Address (District where patient lives)	District name	
Admission date (in AD)	DD/MM/YYYY	
Unit location	Ward, HDU, ICU	
Randomization Arm	Group A, Group B	

C. Clinical and Laboratory Questionnaire (Standard of Care Evaluation for all participants)

No.	Questions/ Variables	Data / value	Unit
	BASELINE CLINICAL DATA		
C1	Duration of fever	No. of days	
C2	Clinical Diagnosis		
C3	System involvement	RTI, GI, UTI, CVS, CNS, SSTI, Blood, Sepsis, Lymphatic, Other	
C4	Comorbidities	None, DM, HTN, COPD/chronic pulmonary dis., CHF, MI, PVD, CLD/Liver failure, PUD, CVA, hemiplegia, dementia, CTD, AIDS, solid tumor, lymphoma, leukemia, other	
C5	Temperature in Celsius (baseline at presentation)		Celcius
C6	BP (baseline at presentation)		mmHg
C7	Pulse (baseline at presentation)		per min.
C8	Resp rate (baseline at presentation)		per min.
C9	SaO2 (baseline at presentation)		%
C10	WBC (baseline at presentation)		per cumm
C11	ANC (baseline at presentation)		per cumm
C12	ALC (baseline at presentation)		per cumm
C13	AEC (baseline at presentation)		per cumm
C14	Hgb (baseline at presentation)		g/dL
C15	Plt		per cumm
C16	CRP		
C17	ESR		per hour
C18	Cr		
C19	ALT		
C20	AST		
C21	UA: pus cells		
	MICROBIOLOGICAL DATA		
M1	Blood culture result	Organism name	
M2	Urine culture result	Organism name	
M3	Sputum culture result	Organism name	
M4	Pus culture	Organism name	
M5	GeneXpert	Positive/negative	
M6	Dengue serology	NS1, IgM, IgG	
M7	Scrub typhus	Positive/negative	

M8	Malaria RDT	P falciparum, P vivax, other	
M9	Kala Azar	Positive/negative	
M10	Brucella	Positive/negative	
M11	MTB	Positive/negative	
M12	Leptospirosis	Positive/negative	
M13	Hepatitis A	IgM	
M14	Hepatitis E	IgM	
M15	Hepatitis B	HBsAg	
M16	Hepatitis C	HBC Ab	
M17	HIV		
M18	Other test results (……)		
M19	Other test results (……)		
M20	Other test results (……)		
	RADIOLOGICAL DATA		
R1	X-ray finding	Consolidation, Infiltrates, Pleural effusion, CHF, ARDS, other	
R2	CT Chest	Consolidation, Infiltrates, Pleural effusion, CHF, ARDS, other	
R3	CT Abdomen	Hepatomegaly, pancreatitis, Cholecystitis, Gall stone, Ascites, appendicitis, typhlitis, intrabdominal abscess, lymphadenopathy, other	
R4	USG Abdomen	Hepatomegaly, pancreatitis, Cholecystitis, Gall stone, Ascites, appendicitis, typhlitis, intrabdominal abscess, lymphadenopathy, other	
R5	CT/MRI skull	Abscess, Meningitis, Encephalitis, parameningeal focus, other	

R6	Echocardiogram	Valvular vegetation, Pericardial effusion, other	
	ADDITIONAL SEROLOGICAL EVALUATIONS (for Group B patients only)	**Results**	
D1	Murine typhus	Positive, negative, NA	
D2	Bartonella	Positive, negative, NA	
D3	Q-fever	Positive, negative, NA	
D4	Chikungunya	Positive, negative, NA	
D5	Zika	Positive, negative, NA	
D6	Japanese B encephalitis	Positive, negative, NA	
D7	West Nile virus	Positive, negative, NA	

D. Severity and Comorbidity indices

i. qSOFA score

Altered mental status GCS <15	Yes	No
Respiratory rate ≥22/min	Yes	No
Systolic BP ≤100 mmHg	Yes	No
Total Score		

ii. Updated Charlson Comorbidity index score

Index	**Score**
CHF	2
Chronic pulmonary disease	1
Connective tissue disease	1
Dementia	1
DM with end organ damage	1
Liver disease- Mild Moderate to severe	2 4
Renal disease- moderate to severe	2
Hemiplegia	2

Tumor without metastasis Tumor with metastasis	2 6
Leukemia	2
Lymphoma	2
AIDS	2
Total Score	

E. Antimicrobials Dose and Durations

	Antimicrobial agent used	**Route**	**Total Dose per day (Grams)**	**Total No. of days used**
AM1		PO, IV	grams	No. of days
AM2				
AM3				
AM4				
AM5				
AM6	Appropriateness of Antimicrobials used (to be determined by an independent physician)	Yes, No, Partially		

F. Outcomes

	Outcome variable	**Results**
	Outcome at the end of hospitalization	Discharge- complete recovery, Discharge- partial recovery, Death
	Composite outcome at the end of hospitalization	Good Outcome (complete plus partial recovery) Poor outcome (Death)