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Enhancing vaccine acceptance, hygiene, and trust among community health workers through a microbe literacy intervention: a cluster randomized controlled trial in Sargodha District, Pakistan

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Abstract

A limited understanding of how microbes are transmitted contributes to weak hygiene practices, low immunization uptake, and mistrust of frontline health services in many low-resource settings. This study evaluated whether a microscope-based "germ-watching" community education program (the Microbe Literacy Initiative, MLI), delivered by Lady Health Workers (LHWs), improves hygiene practices, vaccination attitudes and intentions, routine immunization, and trust in LHWs among mothers and female caregivers of children aged three years and under. A two-arm, parallel-group cluster randomized controlled trial was conducted across twelve Union Council (UC) clusters of Sargodha District, Punjab, Pakistan, between 1 May and 31 August 2024, with clusters allocated 1:1 to the intervention or to routine services (six clusters and approximately 600 households per arm; approximately 1,200 households in total). Trained LHWs used portable microscopes to show mothers the microorganisms in their own household samples during a single 120-minute workshop. Hygiene practices, vaccination attitudes and intentions, and trust in LHWs were measured by structured, interviewer-administered surveys at baseline and endline; routine immunization was assessed both from facility-recorded OPV3 coverage and by self-reported completion of the age-appropriate schedule. Because randomization was at the cluster level, effects were estimated at the cluster level using a difference-in-differences comparison of the six intervention and six control clusters, with 95% confidence intervals (CIs); intracluster correlation coefficients (ICCs) were estimated from the baseline data. After accounting for clustering, the intervention was associated with significant improvements in hygiene and immunization: self-reported handwashing increased more in the intervention arm than in the control arm (child handwashing difference-in-differences +46.8 percentage points [pp], 95% CI 8.4 to 85.3, $p = 0.025$; maternal handwashing +45.0 pp, 95% CI 7.9 to 82.1, $p = 0.025$), as did self-reported completion of the childhood immunization schedule (+56.3 pp, 95% CI 16.2 to 96.5, $p = 0.012$); facility-recorded third-dose oral polio vaccine (OPV3) coverage was greater in intervention clusters (98.5% vs 81.8%). Improvements in trust in LHWs (+14.2 pp, 95% CI −23.7 to 52.0), perceived vaccine safety (+15.8 pp), intention to vaccinate (+14.8 pp), and willingness to recommend vaccination (+34.0 pp) were greater in the intervention arm but did not reach statistical significance once clustering was taken into account, reflecting the limited power of six clusters per arm. Several intervention-arm endline values reached 100%, indicating probable ceiling and measurement-reactivity effects; thus, the findings should be regarded as preliminary. A single, low-cost, microscope-based workshop delivered by LHWs was associated with meaningful gains in household hygiene and childhood immunization; effects on vaccine-related attitudes were suggestive but not statistically robust in this small number of clusters and warrant confirmation in a larger, adequately powered trial. The trial was reported following the CONSORT extension for cluster trials and is registered with ClinicalTrials.gov (NCT07771738).

Keywords

Cluster randomized controlled trial; Germ watching; Health personnel; Hygiene; Immunization; Lady health workers; Microbe literacy; Vaccine hesitancy

1. Introduction

Microbes—including bacteria, fungi, and viruses—are fundamental to human health and to the ecosystems that sustain life, yet they are also responsible for a large share of transmissible diseases [1,2]. Although invisible to the naked eye, they underpin ecological function, agricultural and food systems, and human health at both the molecular and community scales [3]. Public understanding of microbial life nonetheless remains limited, particularly in low- and middle-income settings where beliefs about disease and hygiene are frequently shaped by misinformation. "Microbe literacy" — a working knowledge of microbial life and its implications for health, the environment, and sustainability — has therefore emerged as a practical target for health empowerment and preparedness [4,5,6].

Evidence suggests that microbe literacy can support informed hygiene behavior. In a survey of 30 adults in the United States, Kokkinias and colleagues reported that most respondents recognized the beneficial role of microbes, although misconceptions persisted, and social media was a common information source [7]. In southwestern England, a mixed-methods evaluation of peer-education workshops for secondary and primary school pupils reported improved awareness of microbe and hygiene risks together with self-reported gains in handwashing and environmental cleanliness [8]. Together, these studies indicate that microbe literacy interventions can shift both knowledge and everyday hygiene practices.

Such interventions are strongest in lower-middle-income countries, where infectious disease, antimicrobial resistance, and inadequate sanitation place heavy demands on health systems [9,10]. Everyday decisions about hygiene, antibiotic use, and nutrition are often made with little scientific grounding, and Pakistan is no exception [11]. Because conventional awareness campaigns tend to communicate microbes as an abstraction rather than a tangible experience, they often produce limited and short-lived behavior change [12].

Experiential approaches appear more durable. In a cluster-randomized trial among rural women in southern Punjab, a microscope-based microbe literacy intervention produced measurable and sustained improvements in hygiene, including handwashing and the presence of soap in the home, with effects still evident 16 months later and accompanied by reductions in self-reported illness [13]. In Jamshoro, Sindh, COVID-19 vaccine acceptance among participating parents reached 90.7%, compared with just over 50% among nonparticipants [14]. Beyond disease prevention, microbe literacy has also been linked to gains in critical thinking and scientific reasoning relevant to long-term health resilience [15].

Women are central to household hygiene and caregiving, and engaging them directly with the microbial world may foster more supportive attitudes toward hygiene and scientific health interventions. To date, the Microbe Literacy Initiative has not been evaluated in Punjab—a province that accounts for a large share of Pakistan's population and economy—where improvements in health behavior could have substantial aggregate effects. This trial therefore evaluated a microscope-based, lady health worker (LHW)-delivered microbe literacy program in Sargodha District. We hypothesized that, relative to control clusters, intervention clusters would show greater improvements in hygiene practices, attitudes and intentions toward vaccination, routine immunization coverage, and trust in LHWs.

2. Methods

2.1. Study design, setting, and reporting

We conducted a two-arm, parallel-group cluster randomized controlled trial in Sargodha District, Punjab, Pakistan, to assess the effects of the Microbe Literacy Initiative on community knowledge, hygiene behavior, vaccination attitudes, immunization coverage, and trust in LHWs. The Union Council was the unit of randomization, and the household was the unit of analysis. Twelve urban and rural UCs were included, each contributing a cluster of approximately 100 households with a child aged three years or younger. The trial is reported in accordance with the CONSORT 2010 statement and its extension for cluster randomized trials; a completed checklist and a participant flow diagram are provided as supplementary material.

The trial is registered with ClinicalTrials.gov (NCT07771738). Its primary outcomes and analytic approach follow those of a previously published, peer-reviewed randomized evaluation of the same microscope-based microbe literacy intervention in rural Punjab [13], and the study protocol received a priori approval from the institutional review board.

2.2. Ethics considerations

The study was approved by the relevant institutional review board (No. NMDC/DRC/225/09/063-ERC) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and from a parent or guardian for participants aged under 18 years.

2.3. Participants and eligibility

Eligible participants were mothers or female caregivers who were permanent residents of the study area, had at least one child aged under three years, and provided written informed consent. Caregivers aged 14 years and older were eligible, reflecting the local reality that some primary caregivers are adolescents; for participants under 18, consent was obtained from a parent or guardian in line with the approving committee's requirements for minors. Households already participating in a similar intervention, caregivers unable to take part because of physical or psychological constraints, and those intending to relocate during the study period were excluded to limit contamination and loss to follow-up.

2.4. Randomization and allocation

The twelve clusters were first profiled on key demographic characteristics (household size, maternal education, income, and baseline immunization indicators). Restricted, covariate-constrained randomization was then used to allocate six clusters to the intervention arm and six to the control arm so that the two arms were balanced on these cluster-level characteristics. The allocation sequence was generated by a team member who was not involved in recruitment or data collection. Because the intervention was delivered openly in a community setting, participants and LHWs could not be blinded;

however, data entry and statistical analysis were carried out by staff who were blinded to the allocation.

2.5. Intervention and comparison

The workshop followed a three-stage learning process: Participants first agreed that microbes invisible to the eye were real, then considered practical measures to keep microbes out of the body, and finally discussed the role of vaccines in protecting against microbes that enter the body. Vaccination was therefore addressed within the same single session, and the session was facilitated throughout by the community's own Lady Health Worker.

In the MLI model, groups of 25–30 participants first used simple magnifying glasses before being introduced to a biological microscope offering at least 400× magnification. Participants placed small samples from their own environment (for example, unsafe water, spoiled food, or animal waste) on the microscope, focused them themselves, and observed the diversity and movement of microorganisms in a single drop of water, while monitoring allowed the whole group to view the same field. Each session lasted 120 minutes. Intervention clusters received this single workshop, delivered by LHWs trained to operate portable microscopes and to use participatory teaching methods, with microorganisms demonstrated from water samples the women had collected in their own areas. The control clusters continued to receive routine LHW services and standard national health messaging, with no microscope-based session during the study period.

2.6. Outcomes

The primary outcomes were the between-arm differences at endline in self-reported handwashing practices and in routine childhood immunization, the latter assessed both as facility-recorded OPV3 coverage and as self-reported completion of the age-appropriate schedule. Secondary outcomes were trust in LHWs, awareness of vaccine-preventable diseases, perceived vaccine safety, intention to vaccinate, and willingness to recommend vaccination. Outcomes were measured through structured, interviewer-administered questionnaires at baseline and at endline (immediately after the intervention period); the instrument was pilot tested before use, and facility immunization records were used to corroborate self-reported coverage.

2.7. Sample size

With twelve clusters (six per arm) and approximately 100 households per cluster, the trial was designed to detect a clinically meaningful between-arm difference in the primary outcomes with 80% power at a two-sided α of 0.05, allowing for clustering; assuming a control-arm proportion of the primary outcome of approximately 75% and an intracluster correlation coefficient (ICC) of 0.10—which is consistent with the ICCs estimated from the trial's own baseline data (0.09–0.12 for the hygiene outcomes)—the design effect was approximately $1 + (100 - 1) \times 0.10 \approx 10.9$. Under these assumptions, the trial had approximately 80% power at a two-sided α of 0.05 to detect an absolute between-arm difference of approximately 20–25 percentage points in the primary outcome; smaller differences would not be reliably detected. The large average cluster size relative to the small number of clusters yields a high design effect, which limits precision and is addressed in the limitations.

2.8. Data analysis

Analyses were conducted at the household and cluster levels. Baseline characteristics were summarized descriptively by arm. Because randomization was at the cluster level and only six clusters were available per arm, the primary analysis was a cluster-level difference-in-differences comparison: each cluster's outcome proportion was computed at baseline and endline, and the mean baseline-to-endline change in the six intervention clusters was compared with that in the six control clusters using a two-sample t test, yielding a difference-in-differences estimate with a 95% CI. This approach is robust with a small number of clusters and adjusts for the baseline imbalance between arms. Intracluster correlation coefficients (ICCs) were estimated from the baseline data by one-way analysis of variance.

Individual-level generalized estimating equations and mixed-effects logistic models were also examined; however, because several intervention-arm endline proportions were 100%, these models were affected by perfect separation and were not used for inference. A two-sided $p \le 0.05$ was considered to indicate statistical significance. Associations between potential confounders (maternal education, occupation, and household income) and trust in LHWs were examined using chi-square tests.

3. Results

Baseline and endline surveys were completed in all twelve clusters (approximately 600 households per arm at each timepoint). Because randomization was at the cluster level and only six clusters were available per arm, effects were estimated at the cluster level using a difference-in-differences (DiD) comparison, which also accounts for the baseline imbalance between arms. After accounting for clustering, the intervention was associated with significant improvements in hygiene and immunization, whereas improvements in vaccine-related attitudes did not reach statistical significance.

3.1. Primary outcomes: hygiene and immunization

Self-reported child handwashing increased from 54.5% to 100.0% in the intervention arm but remained near 76–77% in the control arm (DiD +46.8 pp, 95% CI 8.4 to 85.3, p = 0.025); maternal handwashing showed the same pattern (+45.0 pp, 95% CI 7.9 to 82.1, p = 0.025). Self-reported completion of the childhood immunization schedule increased from 26.8% to 90.0% in the intervention arm versus 53.2% to 60.0% in the control arm (DiD +56.3 pp, 95% CI 16.2 to 96.5, p = 0.012). The primary outcome estimates are summarized in Table 1. Facility-recorded third-dose oral polio vaccine (OPV3) coverage was higher in the intervention clusters (98.5%) than in the control clusters (81.8%; Figure 1).

Table 1. Primary outcomes: cluster-level difference-in-differences (DiD) for hygiene and immunization.

Outcome	Baseline I/C (%)	Endline I/C (%)	DiD, pp (95% CI)	*p* Value	ICC
Child handwashing (always)	54.5/77.3	100.0/76.0	+46.8 (8.4 to 85.3)	0.025	0.09
Maternal handwashing (always)	59.8/84.8	100.0/80.0	+45.0 (7.9 to 82.1)	0.025	0.12
Full immunization (self-reported)	26.8/53.2	90.0/60.0	+56.3 (16.2 to 96.5)	0.012	0.17

* The values are percentages of households by arm (I, intervention; C, control); n ≈ 600 per arm at each timepoint. ** DiD is the between-arm difference in the baseline-to-endline change, estimated at the cluster level (six clusters per arm) with a 95% CI. *** The ICC is the intracluster correlation coefficient for the outcome at baseline. **** Facility-recorded OPV3 coverage (98.5% vs 81.8%) is reported descriptively in the text. ***** CI, confidence interval; DiD, difference-in-differences; pp, percentage points.

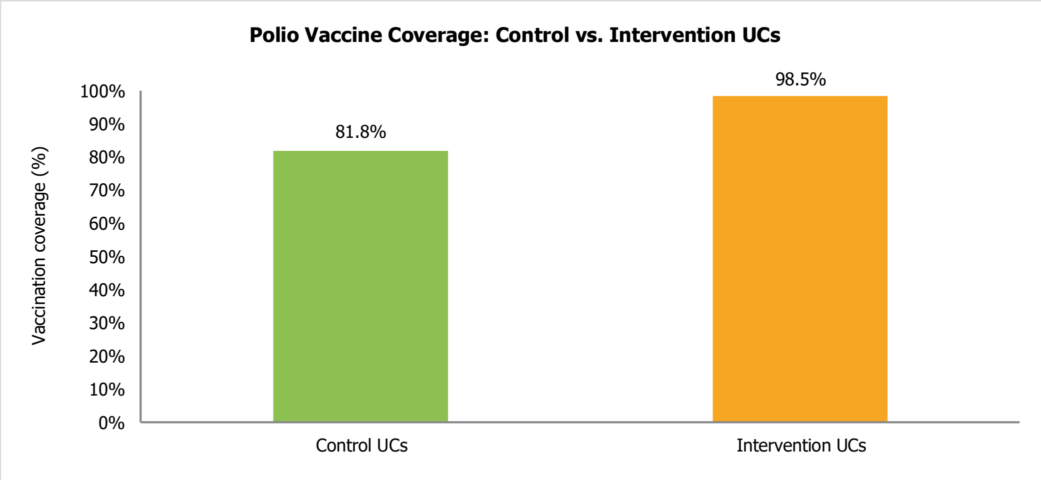

Figure 1. Third-dose oral polio vaccine (OPV3) coverage in control versus intervention Union Councils from facility records.

3.2. Secondary outcomes: attitudes, trust, and knowledge

Vaccine-related attitudes and trust improved more in the intervention arm than in the control arm, but none reached statistical significance once clustering was taken into account (Table 2). Trust in LHWs (DiD +14.2 pp, 95% CI −23.7 to 52.0, $p = 0.39$), perceived vaccine safety (+15.8 pp, $p = 0.36$), intention to vaccinate (+14.8 pp, $p = 0.38$), and willingness to recommend vaccination (+34.0 pp, $p = 0.10$) all had wide confidence intervals that included the null, reflecting the limited power of six clusters per arm. Knowledge that germs cause infectious disease increased similarly in both arms (74.7% vs 75.3% at endline; DiD +5.7 pp, $p = 0.77$), indicating that there was no differential effect.

Table 2. Secondary outcomes: cluster-level difference-in-differences (DiD) for attitudes, trust, and knowledge.

Outcome	Baseline I/C (%)	Endline I/C (%)	DiD, pp (95% CI)	*p* Value	ICC
Trust in LHWs (high)	68.0/62.2	100.0/80.0	+14.2 (−23.7 to 52.0)	0.39	0.01
Perceived vaccine safety	66.0/61.8	100.0/80.0	+15.8 (−23.9 to 55.6)	0.36	0.00
Intention to vaccinate (very likely)	74.2/69.0	100.0/80.0	+14.8 (−24.7 to 54.3)	0.38	0.00
Recommend vaccination	48.5/42.5	100.0/60.0	+34.0 (−8.7 to 76.7)	0.10	0.02
Germs cause disease (knowledge)	38.8/45.2	74.7/75.3	+5.7 (−37.1 to 48.4)	0.77	0.01

[*] Values are percentages of caregivers by arm; n ≈ 600 per arm at each timepoint. [**] DiD is the between-arm difference in the baseline-to-endline change, estimated at the cluster level with a 95% CI. [***] None of the secondary outcomes reached the $p < 0.05$ threshold after accounting for clustering. [****] ICC, intracluster correlation coefficient; CI, confidence interval; DiD, difference-in-differences; LHWs, Lady Health Workers; pp, percentage points.

3.3. Data quality

Several intervention-arm endline proportions reached exactly 100% (600 of 600 respondents), and some control-arm attitudinal values shifted in ways not expected in an untreated group (for example, trust and perceived safety rose to 80%, and one perception measure fell sharply). These patterns are consistent with ceiling and measurement-reactivity effects and possible measurement inconsistency at endline, and they prevented the use of individual-level logistic models because of perfect separation. The primary findings should therefore be regarded as preliminary and be verified against the original survey records before firm conclusions are drawn.

3.4. Confounders

In an exploratory analysis, maternal education was associated with trust in LHWs ($p < 0.05$), whereas maternal occupation ($p = 0.275$) and household income ($p = 0.110$) were not (Table 3).

Table 3. Association between potential confounders and trust in lady health workers.

Potential Confounder	Association with Trust in LHWs	*p* Value
Maternal education level	Significant	< 0.05
Maternal occupation	Not significant	0.275
Household income level	Not significant	0.110

* *p* values are from chi-square tests of association with trust in LHWs. ** The complete contingency table is provided as supplementary material, with cell counts summing to the analytic sample. *** LHWs, Lady Health Workers.

4. Discussion

After accounting for clustering, this cluster-randomized trial revealed that a single, microscope-based, germ-watching workshop delivered by LHWs was associated with significant improvements in household hygiene and childhood immunization. Improvements in vaccine-related attitudes and trust were greater in the intervention arm than in the control arm but did not reach statistical significance, with only six clusters per arm. The pattern for the primary outcomes is consistent with the idea that letting caregivers see microbes for themselves converts abstract health messages into a concrete, memorable experience that translates into household behavior, while the attitudinal findings remain inconclusive in this small trial.

The improvement in handwashing aligns with earlier hygiene trials in Pakistan and comparable settings. Luby and colleagues reported that intensive handwashing promotion with plain soap reduced the incidence of diarrhea among young children by approximately half in high-risk Pakistani communities [16]. A WHO evidence review of eleven handwashing trials revealed an overall 31% reduction in childhood diarrhea, with one day-care trial reporting a two-thirds reduction [17], and broader syntheses suggest that handwashing with soap can lower diarrhea risk by approximately 40–48% [18]. Our findings are also consistent with those of the original microbe literacy trial in southern Punjab, in which a microscope-based intervention improved women's hygiene scores and increased the presence of household soap at the 16-month follow-up [13].

The gains in vaccine confidence and intention echo those of other maternal education interventions. Evidence from low- and middle-income settings suggests that caregiver-focused vaccine education and community-based communication interventions can improve vaccine confidence and childhood immunization uptake by addressing knowledge gaps, misinformation, and trust-related barriers [19]. In rural Sindh, a quasi-experimental study reported that a structured education campaign increased complete immunization from 63.0% to 74.0% by 16 weeks of age [20]. Community-engagement trials for COVID-19 vaccination have shown a similar direction of effect, with personalized mobilization increasing willingness and, at one site, actual uptake by approximately 17 pp [21]. Our effect estimates sit at the upper end of this range, which we interpret cautiously given the design limitations noted below.

Improvements were seen across education and income strata, suggesting that the approach does not depend on literacy: because it relies on direct observation rather than text, charts, or technical explanation, it may be equally accessible to families regardless of schooling. These gains in hygiene, trust, and vaccine-related behavior are plausibly linked to a lower burden of childhood infection, which is itself an important upstream contributor to malnutrition in low-resource settings [22]. We did not measure growth, anthropometry, or malnutrition in this trial; therefore, the connection to intergenerational malnutrition is a hypothesized pathway supported by prior studies [13] rather than an outcome of the present study, and we frame it as such.

Several important limitations temper these findings. First, the trial included only six clusters per arm, which yields wide confidence intervals and limited power; this is the main reason that the attitudinal outcomes did not reach significance despite sizeable point estimates, and their status should be regarded as inconclusive rather than negative. Second, most outcomes were self-reported and were, in part, collected by the same LHWs who delivered the intervention; thus, social desirability bias, courtesy bias, and Hawthorne effects are likely and may inflate the intervention-arm estimates. Third, several intervention-arm endline values reached exactly 100%, and some control-arm values moved in unexpected directions, indicating probable ceiling and measurement-reactivity effects and possible measurement inconsistency at endline; these values prevented individual-level modeling because of perfect separation, and the primary results should be verified against the source survey records. Fourth, there was a baseline imbalance between arms, which the difference-in-differences analysis addresses but cannot fully eliminate. Fifth, concurrent Supplementary Immunization Activities (mass polio campaigns) took place during the study window, and although they reached both arms, complicated attribution, OPV3 was ascertained from facility records that may have been subject to misreporting. Finally, endlines were measured immediately after the intervention; thus, durability is unknown, and the single-district setting limits generalizability. Adequately powered, multidisciplinary trials with more clusters, blinded and objective outcome measures, and longer follow-up periods are needed to confirm these findings.

For policy and practice, these results suggest that microbe visualization could be explored as a low-cost addition to routine LHW outreach to strengthen hygiene and routine immunization, particularly in low-literacy or underserved communities and in persistently high-risk polio areas, where the primary outcome gains observed here would be most valuable. No effect on vaccine-related attitudes and trust has yet been established and should not be assumed. These are preliminary signals rather than recommendations for scale-up, and they should be confirmed in larger, adequately powered, multidistrict trials before wide adoption.

5. Conclusions

A single, low-cost, microscope-based workshop delivered by LHWs was associated with significant improvements in household hygiene and childhood immunization in a low-resource setting after accounting for the clustered design. The effects on vaccine-related attitudes and trust pointed in the same direction but were not statistically significant in this small trial and remain inconclusive. Given the reliance on self-reports, the ceiling effects observed at endline, and the concurrent immunization campaigns, the effect sizes should be read with caution, and the results should be treated as preliminary. Microbe visualization is nonetheless a promising, inexpensive, and scalable candidate for strengthening community hygiene and immunization, and it now warrants confirmation in an adequately powered, multidistrict trial with more clusters, verified data, blinded or objective outcome measures, and longer follow-up.

Supplementary materials: The following supporting information can be accessed through the embedded link: Supplementary Figure S1. CONSORT flow diagram (cluster randomized trial).

Author contributions: Conceptualization, MT, AI, AA, KSV, TB, and UAK; methodology, MT, TB, and UAK; software, MT, AI, and TB; validation, AI, AA, and UAK; formal analysis, MT, and TB;

investigation, MT, AI, AA, KSV, and TB; resources, MT; data curation, TB, and UAK; writing—original draft preparation, AI, AA, KSV, TB, and UAK; writing—review and editing, MT; visualization, MT, and TB; supervision, MT; project administration, MT; funding acquisition, MT. All authors have read and agreed to the published version of the manuscript.

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Ethics statement: The study was approved by the relevant institutional review board (No. NMDC/DRC/225/09/063-ERC) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and from a parent or guardian for participants aged under 18 years. The trial is registered with ClinicalTrials.gov (NCT07771738).

Consent to participate: Not applicable.

Data availability: The data supporting this study's findings are available from the corresponding authors, Muhammad Tariq and Usman Ali Khan, upon reasonable request.

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