of Burkholderia mallei, essential for the bacterium’s survival, proliferation, and evasion of host adaptive immune responses (10). Our findings suggest that an approach combining culture, 16S rDNA sequencing, and multilocus sequence typing be considered for the accurate identification of uncommon bacterial infection.

This study was supported in part by a High Impact Research Ministry of Higher Education Grant (no. E00013-20001), the Naval Medical Research Center–Asia, and the US Department of State, Biosecurity Engagement Program (NAMRU: J-55025-75053).

References

1. Coenye T, Vandamme P, Govan JRW, Lipuma JJ. Taxonomy and identification of the Burkholderia cepacia complex. J Clin Microbiol. 2001;39:3427–36. http://dx.doi.org/10.1128/JCM.39.10.3427-3436.2001

2. Coenye T, Laevens S, Willems A, Ohlen M, Hannant W, Govan JRW, et al. Burkholderia fungorum sp. nov. and Burkholderia caledonica sp. nov., two new species isolated from the environment, animals and human clinical samples. Int J Syst Evol Microbiol. 2001;51:1099–107. http://dx.doi.org/10.1099/00207713-51-3-1099

3. Gerrits GP, Klaassen C, Coenye T, Vandamme P, Meis JF. A novel Legionella species isolated from the environment and clinical cases. Int J Syst Evol Microbiol. 2002;52:2855–8. http://dx.doi.org/10.1099/00207713-52-5-2855

4. Zhang R, Ran Y, Dai Y, Yang H, Zhang H, Lu Y, et al. Infectious granuloma caused by Burkholderia fungorum confirmed by laser-capture microdissection and polymerase chain reaction. Br J Dermatol. 2014;171:1261–3. http://dx.doi.org/10.1111/bjd.13094

5. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement (M100–S24). Wayne (PA): The Institute; 2014.

6. Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, LiPuma JJ. Expanded multilocus sequence typing for Burkholderia mallei. J Clin Microbiol. 2001;39:3427–36. http://dx.doi.org/10.1128/JCM.39.10.3427-3436.2001

7. Sam IC, Karunakaran R, Kamarulzaman A, Ponnampalavanar S, Syed Omar SF, Ng KP, et al. A large exposure to Naegleria fowleri amebae in Karachi’s water supply, Pakistan. J Clin Microbiol. 2001;39:3427–36. http://dx.doi.org/10.1128/JCM.39.10.3427-3436.2001

8. Nierman WC, DeShazer D, Kim HS, Tettelin H, Nelson KE, Feldblyum T, et al. Structural flexibility in the Burkholderia mallei genome. Proc Natl Acad Sci U S A. 2004;101:14246–51. http://dx.doi.org/10.1073/pnas.0403306101

Address for correspondence: Sazaly AbuBakar, Tropical Infectious Diseases Research & Education Centre, Block N & O, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; email: sazaly@um.edu.my

Naegleria fowleri
Meningoencephalitis Associated with Public Water Supply, Pakistan, 2014

Najia K. Ghanchi, Erum Khan, Azam Khan, Wali Muhammad, Faisal Riaz Malik, Afia Zafar

Author affiliations: Aga Khan University, Karachi, Pakistan (N.K. Ghanchi, E. Khan, F.R. Malik, A. Zafar); Karachi Water and Sewerage Board, Karachi (A. Khan); World Health Organization, Karachi (W. Muhammad)

DOI: http://dx.doi.org/10.3201/eid2210.151236

To the Editor: Naegleria fowleri, a free-living ameba, causes acute, fulminant, fatal primary amebic meningoencephalitis (PAM) in persons with history of recreational activities in warm freshwater (1,2). During 2008–2009, thirteen case-patients with PAM and no history of recreational water activity were reported from Karachi, Pakistan (3). Since then, PAM caused by domestic water exposure, nasal cleansing by using neti pots, and ablution has been reported globally (4–6). During 2014–2015, the Aga Khan University Hospital clinical laboratory in Karachi confirmed 19 PAM case-patients without history of recreational activities in warm freshwater.

Karachi has a subtropical, arid climate and long summers (March–October). The increasing number of PAM cases might be attributable to rising environmental temperatures and a dysfunctional water supply system in Karachi (7). Data indicating direct evidence of N. fowleri amebae in Karachi’s water supply are limited, but consistent annual reemergence of PAM in patients without history of recreational water exposure raises concerns about Karachi’s water supply.

In August 2014, a previously healthy 34-year-old man living in Karachi and having no recreational water exposure was admitted to the Aga Khan University Hospital with multiple episodes of vomiting, severe headache, and fever. Cerebrospinal fluid culture showed a low glucose level (46 mg/dL [reference 45–80 mg/dL]) and high levels of protein (216 mg/dL [reference 20–40 mg/dL]), erythrocytes (30 cells/mm³ [reference 0–10 cells/mm³]) and leukocytes (1,440 cells/mm³ [reference 0–5 cells/mm³]; 65% lymphocytes and 35% neutrophils). PCR confirmed presence of N. fowleri. The patient died 4 days after admission, and cerebrospinal fluid and blood cultures were negative for bacterial and fungal growth.

We investigated for presence of N. fowleri amebae in domestic water and for the patient’s possible exposure. In September 2014, we collected 23 samples from 2 water treatment plants (plants A and B), their pumping...
stations, and catchment areas (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/10/15-1236-Techapp1.pdf). Plant A supplied 13 of the samples from its water distribution system, which provided water to the patient’s residence and neighborhood mosque (Table). The other 10 samples were from Plant B and its water distribution system (online Technical Appendix Table), for which the local government had initiated additional chlorine enhancement because of previously occurring PAM cases. Plant A had no chlorine enhancement. Real-time PCR, as described (8), confirmed presence of \textit{N. fowleri} amebae in the plant’s water supply distribution. Both plants are monitored for quality control by using World Health Organization water treatment procedures guidelines (http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf). The plants’ water distribution exit points had the highest residual chlorine levels (0.5 ppm) (9), and levels gradually decreased beyond the plants. Residual chlorine was undetectable in plant A’s water distribution to the patient’s residential area (12 km from the plant); however, with plant B’s additional chlorine enhancement stations, chlorine levels were detectable in all households tested.

Residual chlorine and specimen positivity for free-living amebae were inversely correlated. Differences were most noticeable in samples collected from plant A’s distributed water, compared with plant B’s water distribution samples. PCR confirmed \textit{N. fowleri} amebae in 2 water samples collected from the patient’s household overhead storage tanks and neighborhood mosque. The samples, taken from plant A’s distributed water, showed no residual chlorine and a temperature >30°C. Lack of detectable chlorine and water temperature >25°C might have provided favorable conditions for \textit{N. fowleri} amebae to thrive in domestic water (5); water temperatures 25°–40°C are favorable for \textit{N. fowleri} growth. Absence of

---

**Table. Characteristics of 13 water samples collected from water treatment plant A and its distribution system, including water supplied to the apartment and neighborhood mosque of a patient with primary amebic meningitis, Karachi, Pakistan, 2014**

| Water supply               | Sample location                  | Sample type               | Total chlorine, mg/L | Temperature, °C | Culture positivity for FLAs | PCR results for Naegleria fowleri amebae‡ | Distance relationships of water samples |
|----------------------------|----------------------------------|---------------------------|----------------------|----------------|--------------------------|------------------------------------------|----------------------------------------|
| Reservoir§                 | Water from Kinjhar               | Untreated                 | ND                   | 30             | ++                       | –                                        | From reservoir to plant A, <100 km      |
| Water treatment plant A    | Filtration unit¶                 | Treatment underway        | <0.5                 | 29.5           | ++                       | –                                        |                                        |
|                            | Plant A exit point               | Filtered and chlorinated  | 0.5                  | 30             | ++                       | –                                        |                                        |
| Pumping station#           | Pumping station, site 1          | Filtered and chlorinated  | ND                   | 30             | ++                       | –                                        | From plant A to pumping station, ≈11 km |
|                            | Pumping station, site 2          | Filtered and chlorinated  | ND                   | 30             | ++                       | –                                        |                                        |
| Catchment areas:           | Mainline                         | Filtered and chlorinated  | ND                   | 30             | –                        | –                                        | From pumping station to patient’s house, 1 km |
| patient’s apartment        | Underground boring well water**  | Untreated                 | ND                   | 28.5           | +                        | –                                        | Within patient’s house, ≈10 m          |
| and neighborhood           | Underground tank                 | Mixed††                   | ND                   | 28             | +                        | –                                        | Within patient’s house, ≈10 m          |
|                            | Overhead tank                    | Mixed                     | ND                   | 31             | ++                       | +                                        | Within patient’s house, ≈5 m            |
|                            | In-house storage tank‡‡          | Mixed                     | ND                   | 30             | ++                       | –                                        | Within patient’s house, ≈5 m            |
|                            | Bathroom                         | Mixed                     | ND                   | 29             | +                        | –                                        | Within patient’s house, ≈5 m            |
|                            | Neighborhood mosque              | Filtered and chlorinated  | ND                   | 31             | ++                       | +                                        | From patient’s house to mosque, ≈100 m  |

‡FLAs, free-living amebae; ND, not detected; ++, >3 amebae seen with 40x magnification; +, 1–3 amebae seen with 40x magnification; –, no amebae detected.
†Water temperatures of 25°C–40°C are conducive for flourishing of \textit{Naegleria fowleri} amebae.
‡‡Underground and overhead tanks are shared by >100 households, so for continuous water supply, residents keep small tanks in their homes.
the amoebae in plant B’s water suggests the importance of enhanced chlorine pumping at distribution points beyond water treatment plants for maintain residual chlorine in Karachi’s domestic water supply.

Because water supply can be intermittent, underground and overhead storage tanks are essential for Karachi homes. To ensure continuous domestic supply, water is stored in overhead tanks and pumped from tanks into homes as needed. Water storage in tanks perhaps facilitated propagation of \textit{N. fowleri} amoebae in domestic and mosque water. During the summer, ambient temperatures reach 44°C, leading to increased water temperatures in overhead tanks. We found water temperatures up to 34°C, which may facilitate excystation of \textit{N. fowleri} amoebae to infective forms. Slime, dirt, and high ambient temperatures likely explain \textit{N. fowleri} amoebae in mosquito water in Karachi.

Presence of \textit{N. fowleri} amoebae in mosque water is alarming. Ablution (Wudhu) is a ritual performed by Muslims before offering prayers and involves thorough cleaning of mouth, ears, face, arms, feet, and nasal passages, the latter by inhaling water forcefully up the nostrils. Performing this activity with contaminated water could be a communal source for potential outbreaks.

Karachi water supply authorities have initiated chlorine enhancement at various sites beyond plant B, and our findings support the need for this enhancement. We recommend that the government implement measures to maintain appropriate chlorine levels in the domestic water supply and at recreational sites and to develop effective amebae-monitoring programs. The public should use boiled or filtered water for nasal cleansing, regularly clean storage tanks, and add supplemental chlorine to water in homes, especially during the summer.

Acknowledgments

We thank the Karachi Water and Sewerage Board and residents of Karachi for providing water samples.

References

1. Capewell LG, Harris AM, Yoder JS, Cope JR, Eddy BA, Roy SL, et al. Diagnosis, clinical course, and treatment of primary amoebic meningoencephalitis in the United States, 1937–2013. J Pediatr Infect Dis Soc. 2015;4:e68–75. http://dx.doi.org/10.1093/jpids/piu103

2. Yoder JS, Eddy BA, Visvesvara GS, Capewell L, Beach MI. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962–2008. Epidemiol Infect. 2010;138:968–75. http://dx.doi.org/10.1017/S0950268809991014

3. Shakoor S, Beg MA, Mahmood SF, Bandea R, Sriman R, Noman F, et al. Primary amebic meningoencephalitis caused by \textit{Naegleria fowleri}, Karachi, Pakistan. Emerg Infect Dis. 2011;17:258–61. http://dx.doi.org/10.3201/eid1702.100442

4. Yoder JS, Straif-Bourgeois S, Roy SL, Moore TA, Visvesvara GS, Ratard RC, et al. Primary amebic meningoencephalitis deaths associated with sinus irrigation using contaminated tap water. Clin Infect Dis. 2012;55:e79–83. http://dx.doi.org/10.1093/cid/cis626

5. Cope JR, Ratard RC, Hill VR, Sokol T, Causey JJ, Yoder JS, et al. The first association of a primary amebic meningoencephalitis death with culturable \textit{Naegleria fowleri} in tap water from a US treated public drinking water system. Clin Infect Dis. 2015;60:e36–42. http://dx.doi.org/10.1093/cid/civ017

6. Primary amebic meningoencephalitis associated with ritual nasal rinsing—St Thomas, US Virgin Islands, 2012. Clin Infect Dis. 2014;58:iii.

7. Sajjad SH, Hussain B, Khan MA, Raza A, Zaman B, Ahmed I. On rising temperature trends of Karachi in Pakistan. Clim Change. 2009;96:539–47. http://dx.doi.org/10.1007/s10584-009-9598-y

8. Qvarnstrom Y, Visvesvara GS, Sriman R, da Silva AJ. Multiplex real-time PCR assay for simultaneous detection of \textit{Acanthamoeba spp., Balamuthia mandrillaris}, and \textit{Naegleria fowleri}. J Clin Microbiol. 2006;44:3589–95. http://dx.doi.org/10.1128/JCM.00875-06

9. Reed B, Shaw R, Chatterton K. Technical notes on drinking-water, sanitation and hygiene in emergencies. Loughborough (UK): World Health Organization; Water, Engineering and Development Centre; 2013.

Address for correspondence: Afia Zafar, Section of Microbiology, Department of Pathology and Laboratory Medicine, Aga Khan University, Stadium Rd, PO Box 3500, Karachi 74800, Pakistan; email: afia.zafar@aku.edu

Unmet Needs for a Rapid Diagnosis of Chikungunya Virus Infection

Elisa Burdino, Guido Calleri, Pietro Caramello, Valeria Ghisetti

Author affiliation: Amedeo di Savoia Hospital, Torino, Italy

DOI: http://dx.doi.org/10.3201/eid2210.151784

To the Editor: Chikungunya virus (CHIKV) has become a global health problem. Clinical manifestations are not specific and are difficult to differentiate from those of similar viral diseases (e.g., dengue and Zika virus disease). Diagnostic laboratories must be prepared to meet the changing epidemiology of viral diseases. CHIKV infection is currently identified by viral genome detection, using reverse transcription PCR (RT-PCR), viral culture, and serologic testing for IgG and IgM by indirect immunofluorescence (IFA) or ELISA. RT-PCR is most sensitive during the early phase of CHIKV infection (within 5–7 days of symptom onset), but its use is limited by the short viremic phase of the disease. After the acute phase, serologic testing for IgG and IgM is a more accurate indicator of disease.
Naegleria fowleri Meningoencephalitis
Associated with Public Water Supply, Pakistan

Technical Appendix

Technical Appendix Table. Water samples (n = 10) collected from water treatment plant B and its distribution system, including households, Karachi, Pakistan, 2014*

| Water supply          | Sample location                                      | Sample type                           | No. samples | Total chlorine, mg/L | Temperature, °C† | Culture positivity for FLAs | PCR results for Naegleria fowleri amebae‡ | Distance relationships of water samples     |
|-----------------------|------------------------------------------------------|---------------------------------------|-------------|----------------------|-------------------|-----------------------------|--------------------------------------------|---------------------------------------------|
| Reservoir§            | Water from Kinjhar Untreated                         | 2                                     | ND          | NT                   | 26                | ++                          | –                                          | From reservoir to plant B, 100 km           |
| Water treatment plant B| Filtration unit Treatment underway                   | 2                                     | 1           | 28                   | ++                | –                                          | –                                          |                                             |
| Water treatment plant B| Plant B exit point Filtered and chlorinated         | 1                                     | 1           | 24                   | –                 | –                                          | –                                          |                                             |
| Water treatment plant B| Pumping station 1 Filtered and chlorinated           | 1                                     | 0.5         | 27                   | +                 | –                                          | –                                          | From plant B to pumping station, 17.5 km    |
| Water treatment plant B| Pumping station 2 Filtered and chlorinated           | 1                                     | >2.5        | 26                   | –                 | –                                          | –                                          | From pumping station site 1 to pumping station site 2, 3.5 km |
| Catchment areas: consumer households| Household 1, underground storage tank Filtered and chlorinated | 1                                     | 0.5         | 29                   | +                 | –                                          | –                                          | From pumping station site 2 to household 1, 2.03 km |
|                      | Household 1, overhead storage tank Filtered and chlorinated | 1                                     | <0.5        | 34                   | +                 | –                                          | –                                          |                                             |
|                      | Household 2, underground storage tank Filtered and chlorinated | 1                                     | traces      | 30                   | –                 | –                                          | –                                          | From pumping station site 2 to household 2, 0.5 km |

*FLAs, free-living amebae; ND, not detected; NT, not tested; +, 1–3 amebae seen with 40 x magnification; ++, >3 amebae seen with 40 x magnification; –, no amebae detected.
†Water temperatures of 25°C–40°C are conductive for flourishing of Naegleria fowleri amebae.
‡PCR was negative for other pathogenic FLAs such as Balamuthia or Acanthamoeba species.
§Kinjhar Lake, located in Sindh province, Pakistan, is the main reservoir that supplies water to Karachi.
Technical Appendix Figure. Locations of water treatment plants A and B and their distribution systems, water sampling sites, and other sites related to samples collected in Karachi, Pakistan, 2014. Inset shows main reservoir and its distance from sampling sites (~100 km from Karachi). Because of a high number of cases reported before the case in this study, chlorine enhancement was initiated at water treatment plant B but not at plant A. *N. fowleri* was not detected after chlorine enhancement at plant B’s distribution system in this study.