travelers returning from Cuba with a rash, similarly to patients returning from other countries in which dengue fever, chikungunya fever, and Zika virus infection are endemic. Preventive measures, including advice to travelers on proper use of insect repellents, are critical for preventing CHIKV infection.

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References

Inactivation and Environmental Stability of Zika Virus

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To the Editor: Zika virus is an emerging virus that has spread to most countries in Latin America and the Caribbean (1,2). It is transmitted by mosquitoes and through sexual intercourse (3). Most persons infected with Zika virus are asymptomatic or experience mild symptoms (4). However, in a pregnant woman, infection may cause severe pregnancy and birth complications, most notably microcephaly in children infected in utero (5–7). Zika virus infection might also be associated with an increased incidence of Guillain-Barré syndrome (8). Thus, the virus represents a threat to healthcare workers who manage infected patients or diagnostic and researchers who work with infectious virus in laboratories.

Working with Zika virus, a Biosafety Level 2 (BSL-2) pathogen in the European Union, except for the United Kingdom (where it is BSL-3), requires specific safety precautions (9). No inactivation data specific for Zika virus are available (9); consequently, disinfection guidelines are based on protocols to inactivate other flaviviruses. To gain experimental evidence regarding inactivation and disinfection for Zika virus, we determined its susceptibility to various disinfectants and inactivation methods.

To test susceptibilities, we determined the 50% tissue cell infectious dose per milliliter (TCID₅₀/mL) (10) of the Zika virus MR766 strain (1) before and after the virus was exposed to disinfectants or other inactivation procedures (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/9/16-0664-Techapp1.pdf). We then determined the effect of alcohol-based disinfectants on viral infectivity. Using Zika virus stock containing 2.5% fetal calf serum (FCS) mixed 3:10 (vol/vol) with indicated alcohols, we incubated the mixture for 1 minute and then used it for infection (Figure, panel A). All alcohols entirely inactivated virions.
the virus. Complete loss of infectivity was also observed after virus exposure to 1% hypochlorite (often used to inactivate virus in liquid wastes in BSL-2/3 laboratories), 2% paraformaldehyde (used to inactivate virus for subsequent flow cytometry), and 2% glutaraldehyde (often applied to fix virus for subsequent electron microscopy analysis) (Figure, panel A). Thus, routinely used disinfectants and inactivation procedures are sufficient to inactivate Zika virus in laboratory virus stocks. Next, we repeated these experiments in the presence of a high protein load using Zika virus preparations supplemented with FCS in increasing concentrations (10%, 40%, 90%), to mimic virus found in clinically relevant material. Thereafter, dried virus was reconstituted in medium or 70% (vol/vol) isopropanol. Isopropanol inactivated the virus, but dried virus in medium remained infectious even after 84 h of incubation. E) Zika virus was incubated for 5 min at indicated temperatures. Temperatures >60°C inactivated the virus. F) Stocks were adjusted to indicated pH values and incubated for 10 min. pH levels ≤4 or >11 deactivated the virus. G) Finger tips of laboratory gloves were cut off, with or without introducing a hole by pinching with a needle, and put into medium. Glove tips were filled with virus stock and incubated for 90 min at room temperature. All gloves without needle holes were protective against transmission; 2 of 3 gloves with needle holes allowed virus transmission. For detailed experimental description, see online Technical Appendix (http://wwwnc.cdc.gov/EID/article/22/9/16-0664-Techapp1.pdf). DMSO, dimethyl sulfoxide; E, ethanol; G, glutaraldehyde; H, hypochlorite; I, incin, IP, isopropanol; M, medium, M+ medium plus 10 min UV; ND, not dried; PBS, phosphate-buffered saline; PF, paraformaldehyde; TCID$_{50}$, 50% tissue culture infective dose; UV, ultraviolet.

We also assessed the environmental stability of Zika virus to heat and change in pH. The virus was stable at temperatures up to 50°C but lost all infectivity at temperatures
of ≥60°C (Figure, panel E). Thus, virus-contaminated materials such as surgical instruments can be decontaminated by heat. We also found that Zika virus infectivity was highest after adjusting the stock to a pH of ≈9 (Figure, panel F). In contrast, adjusting Zika virus to pH 12 or to ≤pH 4 abrogated infectivity (Figure, panel F).

Finally, we analyzed whether gloves routinely used in BSL-2 laboratories protect against Zika virus. For this, we cut off fingertips of nitrile and latex gloves, filled tips with a Zika virus suspension, and placed them into cell culture plates containing medium. Virus-containing fingertipswere inserted in such a way that diffusion would only occur if the virus passed through the nitrile/latex barrier. As a control, we made a hole of <1 mm in the fingertips. All 3 tested gloves prevented virus diffusion (Figure, panel G). However, if glove integrity was disrupted by a pin, the virus passed through in 2 of 3 cases (Figure, panel G).

We demonstrated that Zika virus is destroyed by classical disinfectants and inactivation methods and that nitrile and latex gloves are protective. We also showed that UV light of a laminar flow hood inactivates Zika virus, but particularly if the virus is in a protein-rich environment, the exposure time range should be in hours rather than in minutes. Although we expected that Zika virus would be inactivated by alcohol and disinfectants, we conducted a thorough experimental verification to exclude uncertainties surrounding work with this emerging pathogen.

References


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ESBL-Producing Strain of Hypervirulent Klebsiella pneumoniae K2, France

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To the Editor: Klebsiella pneumoniae is mainly responsible for hospital-acquired urinary tract infections, bacteremia, pneumonia and intra-abdominal infections. However, since the mid-1980s, K. pneumoniae has also been described as the cause of highly invasive or multi-drug-resistant (MDR) strains had been considered independent (3) until 2014, when extended-spectrum β-lactamase (ESBL)– or carbapenemase-producing hvKP were first identified in China (4). Here we report an ESBL-producing strain of hvKP isolated from a patient in France.

The patient was a 56-year-old woman, born in Algeria, who alternately resided in France and Algeria for several years without travel to any other country. She underwent liver transplant in 2007 for primary biliary cirrhosis. In 2012, she had a routine posttransplant liver biopsy indicating...