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New methods, new math speed detection of drug-resistant malaria

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Researchers at Case Western Reserve University developed techniques to quickly identify evolution of drug resistance in strains of malaria. Their goal is to enable the medical community to react quickly to inevitable resistance and thereby save lives while increasing the lifespan of drugs used against the disease.

Currently, disease monitoring requires months of clinical trials. The new methods can provide more information in just days, and far cheaper.

The investigators have tailored genetic assays and mathematical analysis to uncover and track drug immunity of the deadliest form of the disease, caused by the parasite Plasmodium falciparum. But, the technology could be used for other forms of malaria and other diseases.

The investigators report their work in the online journal *BioMed Central Genetics*.

"Each year an estimated 1 million to 2.5 million children die as a direct result of malaria; a conservative estimate is that one child dies of malaria every 30 seconds," said Peter Zimmerman, a professor of international health at Case Western Reserve School of Medicine. "More accurate surveillance of drug resistance in malaria parasite populations will reduce the number of deaths."

Earlier detection of resistance enables health care workers to adjust treatments sooner, ideally before resistance becomes fully established in

a population and eliminates a drug from use, he explained.

"There is no vaccine for malarial parasites; we depend on drugs and the biggest threat is the parasites continue to evolve resistance," said Carol Hopkins Sibley, a professor of genome sciences at the University of Washington and scientific director of the WorldWide Antimalarial Resistance Network. She was not involved in the research.

"They've come up with a better way to identify resistance," Hopkins Sibley said. "You can look at hundreds or thousands of samples in a day or two. That eliminates a lot of work and expense."

A key, the researchers say, is using a nontraditional mathematical analysis that's proved more accurate than traditional methods.

Zimmerman led the development of assays that take a few drops of blood from a patient and tags molecular markers associated with infection with fluorescent beads. One fluorescent tag locks onto the drug-sensitive form of *P. falciparum*, another tag marks the drug-resistant form.

The assays are sensitive enough to reveal change in a single nucleotide - one location among millions on the parasite genome - that has mutated and made the parasite drug-resistant.

But, because there is such a tiny difference between the strains, and therefore fluorescent signals, traditional analysis failed to provide an accurate picture of who, among 264 volunteers from Papua, New Guinea, was infected and by what.

In a traditional analysis, infections are plotted by strength of fluorescent signal as Cartesian points on a graph. The drug-sensitive infections cluster along the x-axis, the drug-resistant along the y-axis. Those with

both infections would be somewhere in between and those with no infection would be clustered where the axes meet - at zero.

But, even when a standard deviation was introduced to account for crossover among the fluorescent signals, Zimmerman wasn't getting a clean delineation of who was infected and with what.

Former math major Drew P. Kouri, who has since graduated, was working in Zimmerman's lab and took the problem to Peter J. Thomas, assistant professor of mathematics and biology. They found they could produce an accurate picture by plotting the data points using polar coordinates.

"The math behind the method is pretty simple," Thomas said. "The key thing that Drew and I noticed was that the cross talk between signals involved a constant ratio of signals. Polar coordinates naturally involve ratios so we gave it a try."

In this method, the x-axis equals the fluorescence for drug-sensitive infection and the y-axis equals the fluorescence for drug-resistant. The 264 blood samples are plotted as points in between the axes according to strength of signal.

The distance and the angle of each point from zero are calculated, and the information is graphed with the x-axis equal to distance and the y-axis angle. The results: 4 distinct groups that reflect the four possible diagnoses.

Using the polar coordinates analysis, 86 of the 264 samples were reclassified.

Zimmerman said further studies are needed to verify that resistance to a specific drug is associated with specific genetic variants.

Thomas said they could seek funding to produce a computer program that automatically processes data using polar coordinates: "If we set up a web-based data-analysis tool, it could be useful for field researchers without specialized mathematical expertise."

More information: http://www.biomedcentral.com/1471-2156/11/57.

Provided by Case Western Reserve University