



News Release

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Session A110: Late Breaking Abstracts In Disease Susceptibility and Pathogenesis

Sunday, May 18, 2014, 2:00 p.m. – 4:30 p.m.

Location: Room 8 (Upper Level), San Diego Convention Center

E-cigarettes May Boost Resistance of Drug-Resistant Pathogens

ATS 2014, SAN DIEGO — Despite being touted by their manufacturers as a healthy alternative to cigarettes, e-cigarettes appear in a laboratory study to increase the virulence of drug-resistant and potentially life-threatening bacteria, while decreasing the ability of human cells to kill these bacteria

Researchers at the VA San Diego Healthcare System (VASDHS) and the University of California, San Diego (UCSD), tested the effects of e-cigarette vapor on live methicillin-resistant *Staphylococcus aureus* (MRSA) and human epithelial cells. MRSA commonly colonizes the epithelium of the nasopharynx, where the bacteria and epithelial cells are exposed constantly to inhaled substances such as e-cigarette vapor and cigarette smoke.

“The virulence of MRSA is increased by e-cigarette vapor,” said lead investigator Laura E. Crotty Alexander, MD, VA researcher and assistant professor of medicine in pulmonary and critical care at UCSD. Exposure to e-cigarette vapor increased the virulence of the bacteria, helping MRSA escape killing by antimicrobial peptides and macrophages. However, she added, the vapor did not make the bacteria as aggressive as cigarette smoke exposure did in parallel studies her group conducted.

To conduct the e-cigarette vapor experiment, the researchers grew MRSA (USA 300 strain) in culture with vapor concentrations similar to inhalers on the market. They tested first for

biochemical changes in the culture known to promote pathogen virulence and then introduced epithelial cell- and alveolar macrophage-killing assays.

The study was presented at the 2014 American Thoracic Society International Conference.

The researchers looked at five factors that contribute to MRSA virulence: growth rate, susceptibility to reactive oxygen species (ROS), surface charge, hydrophobicity and biofilm formation. In particular, e-cigarette vapor led to alterations in surface charge and biofilm formation, which conferred greater resistance to killing by human cells and antibiotics.

Crotty Alexander said that one possible contribution to the increased virulence of MRSA was the rapid change in pH induced by e-cigarette vapor. Exposure changed the pH from 7.4 up to 8.4, making the environment very alkalotic for both bacterial and mammalian cells. This alkalosis stresses the cells, giving them a danger signal, leading to activation of defense mechanisms. The bacteria make their surface more positively charged, to avoid binding by the lethal antimicrobial peptides produced by human innate immune cells. The bacteria also form thicker biofilms, increasing their stickiness and making MRSA less vulnerable to attack.

These changes make MRSA more virulent. However, when MRSA is exposed to regular cigarette smoke, their virulence is even greater. Cigarette smoke induces surface charge changes 10-fold greater than that of e-cigarette exposure, alters hydrophobicity and decreases sensitivity to reactive oxygen species and antimicrobial peptides. In a mouse model of pneumonia, cigarette smoke exposed MRSA had four-times greater survival in the lungs, and killed 30% more mice than control MRSA. E-cigarette vapor exposed MRSA were also more virulent in mice, with a three-fold higher survival.

Unfortunately, while e-cigarette vapor is increasing bacterial virulence, Crotty Alexander has found that the vapor is also decreasing the ability of human epithelial cells to kill pathogens. “As health care professionals, we are always being asked by patients, “Would this be better for me?” Crotty Alexander said. “In the case of smoking e-cigarettes, I hated not having an answer. While the answer isn’t black and white, our study suggests a response: even if e-cigarettes may not be as bad as tobacco, they still have measurable detrimental effects on health.”

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** Please note that numbers in this release may differ slightly from those in the abstract. Many of these investigations are ongoing; the release represents the most up-to-date data available at press time.*

Abstract 57341

Electronic Cigarette Vapor (ECV) Exposure Decreases Staphylococcus Aureus

Susceptibility To Macrophage And Neutrophil Killing

Type: Late Breaking Abstract

Category: 10.06 - Host Defenses and Pathogenic Mechanisms Related to Respiratory Pathogens (MTPI)

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Abstract Body

Rationale Electronic cigarettes (E-cigarettes) are being promoted as a “breakthrough product” by their aid in smoking cessation. The merits and threats of inhaling E-cigarette vapor (ECV) are uncertain. Some researchers have demonstrated that ECV exposure increases airway resistance and enhances cancerous behaviors of cells. Till now no studies have been examined the relationship between ECV and microbial pathogenesis. Methicillin resistant *Staphylococcus aureus* (MRSA) commonly colonizes the nasopharynx, thus is exposed to inhalational substances. We aimed to investigate the potential effects of ECV on MRSA virulence.

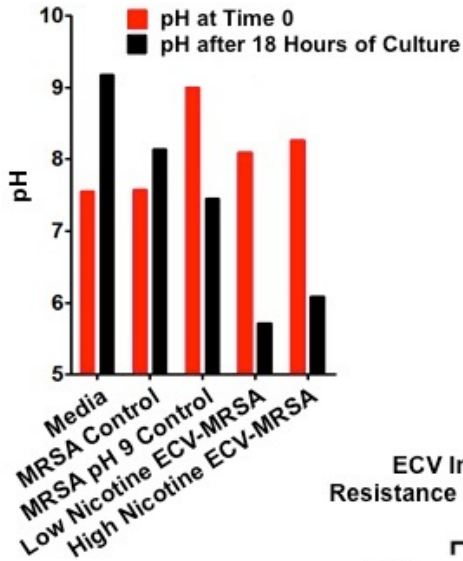
Methods MRSA (USA300 strain) was grown with ECV concentrations similar to what current inhalers use. Growth curves and pH changes were plotted. ECV-MRSA were exposed to H₂O₂ to determine sensitivity to reactive oxygen species (ROS), incubated with poly-L-lysine (PLL) to measure surface charge, and incubated with n-hexadecane to measure hydrophobicity. Neutrophils and macrophages were infected with opsonized MRSA with determination of surviving bacteria via CFU quantification. Biofilm formation was assayed using 5% glucose RPMI, crystal violet exposure, and quantification at 595 nm.

Results: ECV exposure shows modest effects on MRSA growth; however, it induces dramatic pH elevation of the media (pH 8.4) followed by a sustained fall to pH 5.5-5.9 during MRSA growth. ECV decreases the production of MRSA golden pigment. A dose-dependent decrease in PLL-FITC binding was observed in ECV-MRSA. Hydrophobicity and susceptibility to ROS were unchanged. Biofilm formation was augmented in high nicotine ECV-MRSA by 14%, while low nicotine ECV-MRSA had decreased biofilm by 34%. ECV-MRSA had increased survival in both neutrophil and macrophage killing assays compared to controls.

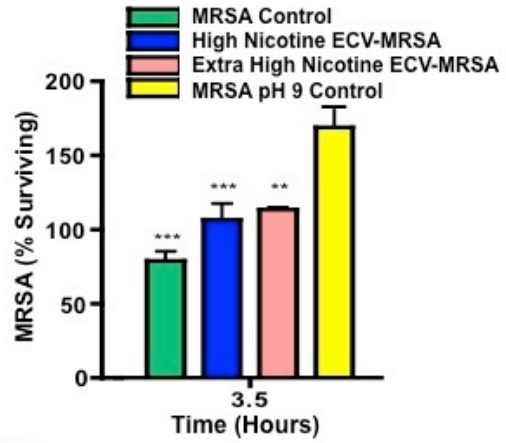
Conclusions: The virulence of MRSA is increased by ECV exposure. ECV induces MRSA to produce acids as a defense mechanism within the first 3 hours of exposure, which raises similarities to their mechanism against nitric oxide - one of the components of innate immune responses to infection. ECV exposed and non-exposed cells have similar fitness under oxidative stress and the same level of hydrophobicity. Surface charge studies demonstrate that ECV drives MRSA to have a more positive surface charge, decreasing susceptibility to antimicrobial peptides. Moreover, ECV exposure up-regulates MRSA biofilm formation, consistent with boosting virulence. In cellular killing assays, ECV-MRSA are able to avoid killing by both macrophages and neutrophils. This is due to ECV

induced changes in surface charge, acid secretion, and biofilm formation. Further studies are ongoing to evaluate which components of ECV are responsible for inducing increased virulence in MRSA.

pH Fall During MRSA Growth in ECV



ECV Increases MRSA Resistance to Macrophage Killing



ECV Increases MRSA Resistance to Neutrophil Killing

