CIGARETTE SMOKING AND EMERGENCE OF MRSA

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ABSTRACT
Cigarette smoking and antibiotic resistance are both a growing menace for the society. In this study Staphylococcus aureus was isolated from human volunteers. The isolates were treated to varying concentrations (10%, 25% and 50%) of Cigarette Smoke Extract (prepared in the lab). The CSE treated isolates were plated in methicillin containing plates. The treated isolates showed profuse growth in the presence of methicillin and was found to be resistant up to 200µg/ml methicillin. The organisms did not grow on methicillin plates when treated with hydrogen peroxide therefore there may be other stress involved other than oxidative stress which is leading to methicillin resistance. This is a firsthand report where we show a direct relationship between cigarette smoking and antibiotic resistance together with the emergence of MRSA.

KEYWORDS: MRSA, cigarette smoking, oxidative stress.

Tobacco has been found to kill nearly 6 million people each year worldwide, more than other life threatening diseases like HIV/AIDS, tuberculosis and malaria combined. More than five millions of those deaths are the result of direct tobacco use while more than 600000 are the result of non-smokers being exposed to second-hand smoke. By 2030 tobacco-related deaths are projected to increase to more than 8 million deaths a year.[1] Cigarettes are the most common form of tobacco used in most parts of the world [WHO] 2006). Cigarette smoking has been correlated with the development of antimicrobial resistance among pathogens apart from the adverse health outcomes associated with it including carcinogenesis[2], promotion of atherosclerosis[3] and chronic lung disease.[4] Cigarette smoking increases the risk of several infectious diseases as well.[5,6] Cigarette smoke has profound effect on the normal respiratory tract microflora including biofilm formation. Smoking-induced biofilm formation restricts the access of antibiotics to pathogens as well as creates an environment conducive to the horizontal transfer of resistant genes, especially in polymicrobial biofilms.[7] Prescribers seem to acknowledge smoking as a risk factor for resistant bacteria since broad-spectrum antibacterial are more frequently prescribed to smokers than never smokers. In addition antimicrobial resistance is a growing menace for human health. In the month of May 2014, the WHO expressed its concern on Earth approaching a “post-antibiotic era in which common infections and minor injuries can kill” [http://www.natureworldnews.com]. Hence, the study of cigarette smoke related antimicrobial resistance among pathogens is of paramount importance along with the understanding of possible threats associated with it before bacteria go on adding the arsenal of resistance to antimicrobials and end up being resistant to a wide range of the antibiotic.

Staphylococcus aureus has a fundamental biological property of colonizing human skin and nasopharynx surviving a variety of environmental niches. It is an opportunistic pathogen notoriously known for its ability to become clinically resistant to antibiotics. Intrinsic resistant mechanisms (acquired by mutation or lateral genetic transfer) can be induced upon exposure to variety of compounds. In our study we hypothesized that the plethora of bioactive compounds in cigarette smoke might affect S. aureus and induce antibiotic resistance. It has been found that the upper respiratory tract contain characteristic microbial communities that exhibit disordered pattern in cigarette smokers.[8] Subsequent reports identified an increased colonization by disease causing organisms in smokers. Another independent study revealed that cigarette smoke increases Staphylococcus aureus biofilm formation via oxidative stress.

In our study we isolated S. aureus from smokers and never smokers including both male and female. The isolates were characterized by morphology of colonies in Baird Parker agar plates, staining and biochemical tests including mannitol fermentation, coagulase production...
and blood haemolysis. The isolates were exposed to Cigarette Smoke extract (CSE) freshly prepared in the laboratory. Effect was tested over a wide range of CSE concentrations i.e. 10%, 25% and 50%. The cells were checked for viability and found that 50% decrease in viability at 25% CSE concentration whereas the colony count was same as control in case of 10% CSE treatment. Therefore 10% CSE was chosen for future work. Log phase cells were treated with CSE (10%) for 24 hrs at 37°C at shaking condition. Post exposure to CSE the cells were grown on methicillin containing plates (10µg/ml). Methicillin resistant S. aureus (MRSA) infection is increasing in many countries in both healthcare and community settings and is a growing menace. Therefore methicillin was chosen for our work. The CSE treated set of isolates showed profuse growth on the methicillin plate whereas the control (not exposed to CSE) did not show growth (Fig 1). CSE treated S. aureus were found to be resistant upto 200 µg/ml concentration of methicillin. Though works have been done to study the effect of cigarette smoking and antibiotic resistance this is a first report of its kind wherein direct relation between cigarette smoking and antibiotic resistance has been studied.

**REFERENCES**


Cigarette smoke contains more than 7300 chemical constituents carcinogens including oxidizing toxic free radicals. To check whether the antibiotic resistance is mediated via oxidative stress, the organisms were treated with hydrogen peroxide (0.25mM, 0.5mM, 1mM and 3mM concentrations) 24 hrs old cultures were centrifuged and the pellet was treated with various concentrations of hydrogen peroxide for 30 mins and plated on methicillin containing plates (10µg/ml) (result not shown). The organisms did not grow on the methicillin plates. Therefore we can say that the induction of antibiotic resistance after CSE treatment is not via the oxidative stress.