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ORIGINAL ARTICLE

Microbiological and molecular identification of bacterial species isolated from nasal and oropharyngeal mucosa of fuel workers in Riyadh, Saudi Arabia

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Abstract This study aimed to determine the bacterial species colonizing the nasal and oropharyngeal mucosa of fuel workers in Central Riyadh, Saudi Arabia on a microbiological and molecular level. Throat and nasal swab samples were obtained from 29 fuel station attendants in the period of time extending from March to May 2014 in Riyadh, Saudi Arabia. Microbiological identification techniques were utilized to identify the bacterial species isolated. Antibiotic sensitivity was assessed for each of the bacterial isolates. Molecular identification techniques based on PCR analysis of specific genomic sequences was conducted and was the basis on which phylogeny representation was done for 10 randomly selected samples of the isolates. Blood was drawn and a complete blood count was conducted to note the hematological indices for each of the study participants. Nineteen bacterial species were isolated from both the nasal cavity and the oropharynx including *Streptococcus thoraltensis*, alpha-hemolytic streptococci, *Staphylococcus hominis*, coagulase-negative staphylococci, *Leuconostoc mesenteroides*, *Erysipelothrix rhusiopathiae* and several others. We found 100% sensitivity of the isolates to ciprofloxacin, cefuroxime and gentamicin. Whereas cefotaxime and azithromycin posted sensitivities of 85.7% and 91.4%, respectively. Low sensitivities (< 60% sensitivity) to the antibiotics ampicillin, erythromycin, clarithromycin and norfloxacin were observed. Ninety-seven percent similarity to the microbial bank species was noted when the isolates were

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compared to it. Most hematological indices recorded were within the normal range. In conclusion, exposure to toxic fumes and compounds within fuel products may be a contributing factor to bacterial colonization of the respiratory tract in fuel workers.

1. Introduction

Air pollution has been a major health concern for decades. Motor exhaust emissions are a complex mixture of gases and particulate matter. Individuals working in stations that provide gasoline were found to be at greater risk of contracting respiratory diseases caused by the inhalation of toxic fumes of gasoline and petroleum products (Tunsaringkarn et al., 2010; De Oliveira et al., 2007). Combustion of vehicular exhaust was found to contain carbon aggregates consisting of tens to thousands of primary carbon particles and mineral particles. Of the particularly harmful substances within fuels are benzene and sulfur dioxide (SO_2) both of which are known to modulate respiratory defenses leaving the respiratory tract susceptible to infections (Mohan et al., 2013).

Aromatic hydrocarbons (particularly benzene) are added to gasoline products as anti-knock agents (i.e. an agent that increases the octane rating of the fuel thus increasing the temperature and pressure necessary for that fuel to undergo auto-ignition). Previously, lead was used for this purpose but is nowadays considered hazardous to health and thus is not used as an anti-knock agent in most countries. Refined petroleum products usually include 2–3% benzene by volume, however in many countries, Saudi Arabia included, contents of benzene in gasoline may reach values ranging between 5% and 7% (Bahadar et al., 2014).

Benzene exposure has many deleterious and toxic effects in general on humans due to both acute and chronic exposure. Benzene is particularly toxic to both the respiratory and hematological systems. Acute exposure to benzene is so irritating to the respiratory system that large concentrations may cause lung edema, hemorrhage and even fatalities (Bahadar et al., 2014). Chronic exposure of the respiratory system to benzene within fuel fumes is also associated with toxic effects on the respiratory system. Apoptotic changes in the parenchymal components of the lungs were noted when rats were exposed to benzene for 7 days (Weaver et al., 2007). Such histological changes may promote the overgrowth of flora or make the system more susceptible to colonization or infection (Bahadar et al., 2014).

Benzene effects on the hematological system have been extensively studied due to its association with hematological malignancies and aplastic anemia. Chronic exposure to benzene is associated with a decrease in hemoglobin (HB), platelet count, and white blood cell (WBC) counts. Neutrophils and mean platelet volume (MPV) in the blood have been reported to be the most likely indices to be affected by benzene exposure in a study that was conducted on Chinese factory workers (Robert Schnatter et al., 2010).

Studies identifying the bacterial pathogens colonizing workers of fuel and gasoline stations are scarce. Most microbiological studies are dedicated to bacteria isolated from areas surrounding fuel stations. Bacterial species found included *Pseudomonas sp*., *Flavobacterium sp*., and *Rhodococcus sp*. (Lu et al., 2006). Other crude oil degrading bacteria such as *Corynebacterium*, *Micrococcus sp*. and *Bacillus sp*. were also isolated from soil samples collected from gasoline and diesel stations (Rahman et al., 2002). Thus far, no study has attempted to analyze and identify the molecular identity of respiratory bacteria isolated from a population exposed to fuel and fuel exhaust contaminants. Like human cells, bacteria have been shown to change in an environment containing toxins or pollutants (Wickham and Atlas, 1988) however there have been virtually no attempts to understand how those exposures would contribute to bacterial pathogenicity.

We conducted this cross-sectional study to determine, both microbiologically and molecularly, the bacterial species from the nasal and oropharynx of fuel workers in Central Saudi Arabia. We also aimed to study their sensitivity to many antibiotics and their possible effects on hematological parameters.

2. Materials and methods

2.1. Sample collection and isolation and testing for antibiotic resistance

This cross-sectional study investigates the effects of gasoline vapors and vehicular exhaust fumes on nasal and nasopharyngeal microbial flora in employees attending fuel stations. Participants were recruited from fuel stations in Riyadh, Saudi Arabia during the period of time between March and May 2014. All the participants were informed about the aim and objectives of the study and approval forms were obtained. The study protocol was reviewed by the Princess Noura Bint Abdul Rahman Research Ethics Committee.

Sterile swabs were used to sample the nares and posterior oropharynx of the participants. We have isolated, identified and assessed antibiotic resistance from 58 samples (where 29 were from the nasal cavity and 29 were from the oropharynx; 2 samples per participant) obtained from the 29 fuel station workers using Vitek® 2 compact system as described by Mezger et al. (2015). The swab samples where then inoculated onto special cards specific for the Vitek® 2 compact. The cards contained wells with reagents and antibiotics with which identification and antibiotic sensitivity could be easily assessed and documented via print out by the system. Alpha hemolysic activity of bacterial isolates was also identified via the Vitek® 2 compact system by pyrosequencing bacterial 16S rRNA gene. This method detects alpha hemolytic abilities and removed the need to inoculate stains onto blood agar plates and interpret their pattern of hemolysis thus leaving room for error in interpretation (Haanperä et al., 2007).

PCR molecular analysis and the phylogeny representation were performed on 10 randomly selected samples from the colonies isolated.

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