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RESEARCH ARTICLE

EXAMINING THE PATTERN OF ANTIBIOTIC SENSITIVITY TEST PROFILE IN *KLEBSIELLA PNEUMONIAE* STRAIN ISOLATED FROM ORAL SWAB OF SMOKELESS TOBACCO CONSUMERS

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ABSTRACT

Klebsiella pneumoniae is found in normal flora of the mouth and can cause irreparable damage to the human body. These pathogens are often resistant to variety of antibiotics and can aggravate illness. This study was undertaken to examine the pattern of Antibiotic Sensitivity of *K. pneumoniae* strain from oral swabs to certain antibiotics. The study is a representation of a cohort population from a socio-economically low background indulging in consumption of smokeless tobacco in Southern Mumbai, India. Oral swabs were collected from smokeless tobacco consumers and non- consumers. The strain was selected through Gram's staining and Maneval's Capsule staining and was further confirmed through Spectrofluorometric analysis and 16rRNA sequencing. Kirby-Bauer's disc diffusion method was used to evaluate the antibiotic sensitivity pattern. Colonies exhibiting a novel fluorescence phenomenon were tested against Ciprofloxacin, Amikacin, Tetracycline and Vancomycin. Twenty-four of the 50 total samples exhibited fluorescence phenomenon. All of the samples were resistant to Vancomycin while 37.5% out of the 24 exhibited resistance to all antibiotics indicating multi-drug resistance. This study may serve as a tool to detect the presence of *Klebsiella sp.* in oral isolates implying host defense mechanism of the oral micro biome reflected in the oral health of the subjects.

INTRODUCTION

Klebsiella pneumoniae is a gram-negative, rod-shaped bacillus from the genus *Klebsiella* and family Enterobacteriaceae (Farver, 2018). Being immobile in nature, this bacterium can be identified by its characteristic to produce a capsule made up of polysaccharide (Azar, 2017). *Klebsiella* pneumoniae grows at an optimum temperature of 37°C and can grow at temperatures varying between 4 to 43°C (Forbes, 2007). The bacterium has been predominantly associated with oral health issues like cavity and abscess formation. In chronic form, it has also been associated being the cause of Parenchymal scarring and Bronchiectasis (Farver, 2018). *Klebsiella spp*. are considered to be important bacterium species associated with multiple infections and are often found to be Multiple Drug Resistant (MDR) human pathogen. *K. pneumoniae* has also been linked to be the prime modulator in the transfer of plasmids and genes that are resistant to antibiotics (Baker, 2019). Hence, the presence of *K. pneumoniae* in the oral microflora should be highlighted. Due to the potency of *K. pneumoniae* strains to become increasingly resistant to drugs, in the recent past, it has become very challenging to treat infection caused by these strains (Paczosa, 2016).

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The aim of this study is to examine the pattern of Antibiotic Sensitivity of *Klebsiella pneumoniae* from oral swabs to antibiotics. The study is a representation of a cohort population from a socio-economically low background indulging in the consumption of smokeless tobacco in Southern Mumbai, India.

MATERIALS AND METHODS

- All the experimental protocol was approved by K.C. College ethics committee.
- All the methods were carried out in accordance with regulations and guidelines of Declaration of Helsinki for involving human participant
- All the study participants provided written informed consent for study participation

- **Study Design:** This study was a community based cross-sectional study that included males from age group between 18-60 years in Southern Mumbai, India.
- **Subjects:** A convenient sampling method was used to recruit smokeless tobacco consumers from a mixed group consisting of taxi drivers, dabbawalas, local vendors and support staff at K.C. College. A total of 50 such people participated in the study. Out of the 50 respondents, 45 were tobacco consumers and 5 were non-consumers.
- **Sample collection:** Unstimulated samples were collected using sterile cotton swabs, by swabbing it all round in the

oral cavity. The swabs were dipped it in a tube containing 1.5ml of saline and further analysed (not later than 24 hours).

- **Microbial Analysis of the sample:** The samples were streaked on Nutrient Agar and Mueller-Hinton agar plates. The plates were incubated at 37° C for 24 hours. *Klebsiella pneumoniae* colonies were picked based on Gram's staining, Maneval's Staining and 16SrRNA genotypic analysis.

RESULTS

Our analysis provides evidence that the oral flora of smokeless tobacco consumers is different than the oral microflora of non-consumers. Out of the 45 tobacco user samples tested, 24 samples (53.33%) confirmed the presence of *Klebsiella pneumoniae* (Fig 1) in the oral swabs. *Klebsiella pneumoniae* strain was confirmed using 16SrRNA sequencing (NCBI nucleotide accession number **KM186520**). The strain was checked against 3 samples of non-tobacco consumers that were used as controls. The controls did not exhibit the presence of this strain. Our findings suggest that the swab samples containing *Klebsiella pneumoniae* showed highest resistance to Vancomycin, Ciprofloxacin and Tetracycline. (Table 1 and Fig 2). All 24 samples have shown 100% resistance to Vancomycin, while a few respondents (37.5%) out of the 24 exhibited resistance to all 4 antibiotics indicating multi-drug resistance (MDR). We observed that 50% of the population showed resistance to Ciprofloxacin while 20% showed resistance to Amikacin and 22.22% showed resistance to Tetracycline (Table 2).

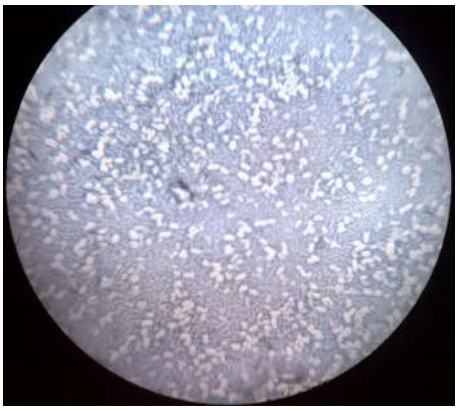

Figure 1. Maneval's Staining showing presence of capsule in the strain

Table 1. Antibiotic resistance pattern of the strain

Antibiotic	Diameter of inhibition	Resistant
Ciprofloxacin (5ug)	≤15mm	Yes
Tetracycline (30ug)	≤11mm	Yes
Amikacin (30ug)	≥20mm	-
Vancomycin (30ug)	0 mm	Yes

Table 2. MDR profile of the subjects

Number of respondents resistant to antibiotics	Antibiotics resistant
3	Ciprofloxacin, Amikacin
12	Ciprofloxacin
10	Tetracycline
9	Ciprofloxacin, Amikacin, Tetracycline and Vancomycin
24	Vancomycin
9	Amikacin

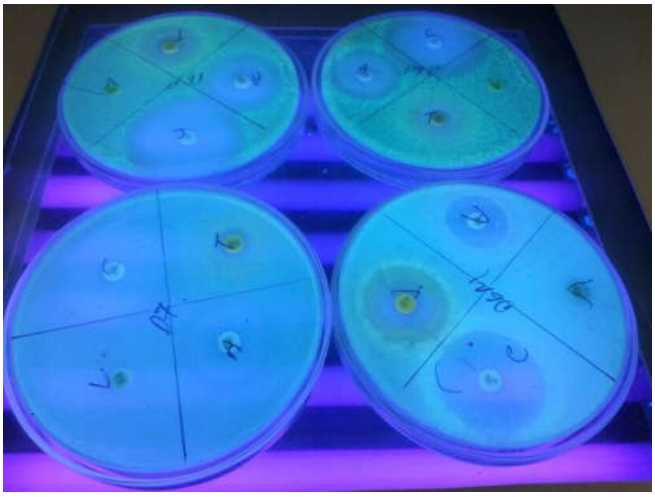

Figure 2. Antibiogram of Pneumoniae from the oral swabs under UV light

DISCUSSION

The use of tobacco in various forms can cause certain changes in the oral cavity because of the different kinds of chemicals they contain. There have been various study reports suggesting that betel quid chewing has a strong association with cancer of the oral cavity, specifically when tobacco is added to quid. Areca derived N-nitrosamines and nicotiana-specific N-nitrosamines derived from tobacco has a high contribution towards oral cancer in betel quid chewers (Saini, 2009). According to one of the surveys conducted, chronic systemic exposure to nicotine could cause disease of the coronary artery, hypertension and acute cardiac ischemic events. Aggravation of these disease has been associated with systemic absorption of sodium and mutagenic chemicals from smokeless tobacco thus contributing to cancer (Wenke, 1984). Few studies have examined that the inverse correlation between normal microflora of tobacco consumers and increased consumption of tobacco indicating that higher the consumption of tobacco cause a loss of the normal microflora. Investigators observed a decreased number of *Streptococcus viridians* which is present in the normal microflora. Researchers also observed presence of environmental bacteria like *Pseudomonas aeruginosa, Klebsiella and Candida albicans* inhabiting the oral cavity. Such subjects were seen to be more prone to develop pre-cancerous conditions thus leading to cancer of the oral cavity with 100% decrease in the normal microflora (Benowitz, 1997).

A previous study by *Bochra Kouidhi et al.* (2011) reported that the oral cavity has been observed to be a reservoir for drug-resistant *Enterococci*. Their findings suggest additional evidence for the continues presence and the ability to adhere in these organisms within the carious lesions. *Enterococci* that possess characteristics of increased drugs resistance, strong biofilm forming abilities and a strong adherence to host cells provides evidence that these three factors may play a pivotal role in *enterococcal* infections. The presence of such pathogen in the dental biofilm in addition to its multi drug resistance ability needs a closer attention and deeper understanding in order to combat the risk for developing systemic diseases caused by *Enterococci* in other areas of the body. In our study, *Klebsiella pneumoniae* was isolated from 24 samples (53.33%) out of 45 samples of oral swabs obtained from Smokeless Tobacco Consumers and compared against the 5 controls obtained from non-consumers.

The study may help to highlight the need for outreach programs that could benefit this section of people about the impact of alteration of the oral micro biome. The ignorance of the respondents to visit the physician or dentist reflects in the negligence of onset of these diseases of these subjects. The subjects need to be made aware of a definite correlation between their oral health and other health parameters. The biochemical nature of fluorescent component in *Klebsiella pneumoniae* remains to be investigated. There have been previous references which say that consumption of tobacco may alter the microenvironment in the oral cavity. It may act as facilitator for non-resident bacteria and may cause changes in the adherence pattern of the micro flora. In our analysis, there must have been such a non-identified factor in oral swabs of tobacco consumers which may have played a role. This study may serve as a tool to detect the presence of Klebsiella species in oral isolates

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