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Comparative Study on the Nasal Bacterial Colonization of Petrol Station Staff and Non-Petrol Station Staff

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ABSTRACT
A total of 224 nasal swabs from 112 petrol station staff and 112 non-petrol station staff were collected and processed bacteriologically using standard techniques to determine bacterial colonization, load, prevalence and sex distribution of the isolates. Bacterial load in form of total aerobic count were 60.56±1.93×10^3 CFU/ml in males and 57.72±2.28×10^3 CFU/ml in females for petrol station staff while 58.82±2.32×10^3 CFU/ml in males and 59.47±1.93×10^3 CFU/ml in females were obtained for non-petrol station staff. The result revealed that there was no significant difference in bacterial count between petrol station staff and non-petrol station staff (P>0.05). The prevalence of the organisms isolated from the study population showed that *Staphylococcus epidermidis* was the most common in petrol station staff 68(60.61%), followed by *Staphylococcus aureus* 36(32.14%), *Streptococcus* species 24(21.43%) and *Proteus mirabilis* 20(17.86%) while *Enterococcus faecalis* was the least organism isolated having 8(7.14%). Also, in non-petrol station staff, *Staphylococcus epidermidis* was the most prevalent 80(71.43%), followed by *Staphylococcus aureus* 32(28.57%), *Proteus mirabilis* 24(21.43%) and *Streptococcus* species 16(14.26%) while *Enterococcus faecalis* was the least 12(10.72%). Frequency of occurrence of the isolates among sex ranged from 4(8.33%) to 32(66.67%) for males and 4(6.25%) to 36(56.25) for females in petrol station staff for *Enterococcus faecalis* and *Staphylococcus epidermidis* respectively, while in non-petrol station staff, it ranged from 4(7.69%) to 36(69.23) in males and 8(13.33%) to 44(73.33%) in females for *Enterococcus faecalis* and *Staphylococcus epidermidis* respectively. The variation in the prevalence rate of the organisms do not differ significantly (P>0.05).

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INTRODUCTION

Microorganisms that colonize the nasal cavity enter an environment quite different from ones encountered by skin organisms. The dark caverns and tortuous passages of the respiratory system are relatively hot and humid and the atmosphere contains less oxygen and more carbondioxide than occurs in air (Nester *et al.*, 2008). Therefore, the human nostril with its constant nourishment and moisture, relative stable pH and temperature and extensive surface upon which to settle, provides a favourable habitat for an abundance of microorganisms. The large and mixed collection of microorganisms adapted to the nasal cavity has variously been called normal resident or indigenous flora (Talaro, 2005; Prescott *et al.*, 2005).

The normal bacteria flora that most commonly colonize the nose are the staphylococci, *Corynebacterium* and streptococci (Brooks *et al.*, 2004; Aneja, 2005) The range of organism that makes up a person's flora is dependent on a number of factors including age, gender, hormonal activity, race, environment, diet and nutrition (Aikpitanyi-Iduitua *et al.*, 2004; Eze *et al.*, 2006). The normal flora can become pathogenic and cause disease if a suitable opportunity arises, such as the transfer of commensals from usual site or habitat to another part of the body where it can establish itself and cause disease, the weakening of a person's natural immunity due to poor health, malnutrition, previous surgery or other underlying disease conditions (Corne *et al.*, 2004; Cheesbrough, 2006).

Petrol, also known as gasoline is one of the lightest products derived from fractional distillation of petroleum. Occupational exposures to this product may occur at any stage from drilling, shipping and refining to use and disposal (Rycroft, 1990; Okoh, 2006). Major exposures may occur both occupationally and environmentally when fuel leaks, spills or otherwise contaminates soil and water (Cook, 1989; Miller and Chang, 2003). Petrol, which is the most widely used petroleum product, is a mixture of mostly low molecular

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weight alkanes and aromatics such as toluene, xylene and benzene. It is highly volatile and its major effects are related to its volatile constituents (Sprince *et al.*, 1994).

Mild poisoning from fumes of petrol occurs in industries where it is used as solvents, in dry cleaning establishments, petrol stations and garages where mechanics are exposed to the vapours. Inhalation of high concentration of petrol may cause sudden loss of consciousness, coma or even death, lower concentration may produce euphobia, flushing of face, mental confusion, difficulty in swallowing and blurred speech and with repeated exposure to fairly high concentration, the patient may develop anorexia and weight loss, headache, nervousness, muscular weakness, polyneuritis and anaemia (Rycroft, 1990; Sprince *et al.*, 1994; d'Abbs and Maclean, 2000).

The nose comes in direct contact with the environment and is thus exposed to fumes and microorganisms in the air. It can harbor potential bacteria pathogens from smoke, soat, dust and petrol fumes from the air resulting to abnormalities in health. However, reduction in host's resistance to infection following exposure to benzene (one of the products of petrol) has been demonstrated (Rushton, 1996). Therefore, the activities of bacteria colonizing the nasal cavity, which otherwise may not be harmful, can be disrupted by petrol fumes obtained through inhalation as a result occupational exposure, resulting to disease condition. Hence, the aim of this study is to determine if inhaling petrol fumes has effect on the bacterial colonization of the nose.

MATERIALS AND METHODS

2.1. Source of Materials:

This study was carried out in Okigwe, a town located at the Northern part of Imo State found in the tropical rain forest zone of Nigeria. The test samples (nasal swabs) were collected from twenty-eight (28) serving petrol station staff in Okigwe metropolis. The volunteers involved both males and females staff. Also, non-petrol station staff volunteers, both male and female were also involved in the screening tests.

2.2. Collection of Nasal Specimen:

A total of 224 subjects, which comprised of 112 petrol station staff, and 112 non-petrol station staff (control). Nasal swabs were collected from subject aseptically using sterile swab sticks which were inserted into a depth of 2cm and rotated five times and then withdrawn. The swabs in their transport medium (plastic tubes) were sent to the laboratory and processed within two (2) hours of collection.

2.3. Determination of Bacterial Load:

Bacterial load was determined as the total viable count (TVC) following the method described by Aikpitanyi-Iduitua *et al.* (2004). 5ml of sterile normal saline was added to each tube of swab stick, agitated and left for 10 minutes for the organisms to disperse and spread out. It was agitated again, and an inoculum, a loopful, from a wire-loop calibrated to deliver 0.01ml was aseptically collected from the suspension of the nasal swab sample in normal saline. The inocula were deposited onto a sterile plate of a nutrient agar (N.A.) and spread evenly with the aid of a sterile glass hockey stick. The inoculated plates were incubated at 37^0C for 24-48h. The same process was repeated using mannitol salt agar (MSA) and MacConkey agar. In each case, plating was done in triplicates.

On establishment of growth, the number of colonies was determined by counting in an electronic colony counter. Counts were taken from plates supporting less than 300 colonies and the total count was recorded with results obtained from the nutrient agar plate cultures.

2.3. Determination of Nasal Bacteria Flora:

Nasal bacteria flora was determined as the type of bacteria which colonized the nostril of the test individuals on analysis described below. Inocula of loopfuls of the nasal suspension prepared as above were cultured by the spread plate method and incubated at 37^0C for 24-48h. On establishment of growth, each cultured plate was examined closely for the presence of distinct colonies from which inocula were taken and sub-cultured in fresh sterile medium. The subcultures were incubated at 37^0C for 24-48h and observed for pure cultures. The pure cultures obtained above were identified using a four step characterization process reported by Fawole and Oso (1988) and Okereke and Kanu (2004).

Results:

The bacteria load in form of total aerobic count as determined tin the present study were $60.56\pm1.93\times10^3$ CFU/ml in males and $57.72\pm2.28\times10^3$ CFU/ml in females for petrol station staff while $58.82\pm2.32\times10^3$ CFU/ml in males and $59.47\pm1.93\times10^3$ CFU/ml in females were obtained for non-petrol station staff (Table 1).

The prevalence of the organisms isolated from the study population showed that *Staphylococcus epidermidis* was the most common in petrol station staff 68(60.61%), followed by *Staphylococcus aureus* 36(32.14%),), *Streptococcus* species 24(21.43%) and *Proteus mirabilis* 20(17.86%) while *Enterococcus faecalis* was the least organism isolated having 8(7.14%). Also, in non-petrol station staff *Staphylococcus epidermidis* was the most prevalent 80(71.43%), followed by *Staphylococcus aureus* 32(28.57%), *Proteus mirabilis* 24(21.43%) and *Streptococcus* species 16(14.26%) while *Enterococcus faecalis* was the least 12(10.72%) (Table 2).

Frequency of occurrence of the isolates among sex ranged from 4(8.33%) to 32(66.67%) for males and 4(6.25%) to 36(56.25) for females in petrol station staff for *Enterococcus faecalis* and *Staphylococcus epidermidis* respectively, while in non-petrol station staff, it ranged from 4(7.69%) to 36(69.23) in males and 8(13.33%) to 44(73.33%) in females for *Enterococcus faecalis* and *Staphylococcus epidermidis* respectively. The variation in the prevalence rate of the organisms do not differ significantly (P>0.05).

Table 1: Determination of the bacterial load (total aerobic count) of the study population.

SEX	Petrol station staff		Non-petrol station staff		
	X±SD	CFU/ML ($\times 10^3$)	X±SD	CFU/ML ($\times 10^3$)	P-VALUE
Male	121.11±3.86 (n=48)	60.56±1.93	117.64±4.63 (n=52)	58.82±2.32	P>0.05
Female	114.66±2.73 (n=64)	57.33±1.37	118.93±3.85 (n=60)	59.47±1.93	P>0.05
Total	117.43±4.56 (n=112)	58.72±2.28	118.33±4.24 (n=112)	59.17±2.12	P>0.05

Key: n = number of subjects, X= mean, SD = standard deviation, CFU/ML = colony forming unit per milliliter, ± = plus or minus.

Table 2: The prevalence of bacteria isolated from petrol station staff and non-petrol station staff.

Isolate	Petrol Station Staff (n=112)	Non-Petrol Station Staff (n=112)
Staphylococcus aureus	36(32.14%)	32(28.57%)
Staphylococcus epidermidis	68(60.61%)	80(71.43%)
Streptococcus species	24(21.43%)	16(14.26%)
Proteus mirabilis	20(17.86%)	24(21.43%)
Enterococcus faecalis	8(7.14%)	12(10.72%)

Key: n = number of subjects, % = percentage.

Table 3: The Sex distribution of the prevalence of the organisms isolated from petrol station staff and non-petrol station staff among sex.

Isolate	Petrol Station Staff (n=112)		Petrol Station Staff (n=112)		
	Male (n=48)	Female (n=64)	Male (n=52)	Female (n=60)	P-Value
Staphylococcus aureus	16(33.33%)	20(31.25%)	12(23.08%)	20(32.33%)	P>0.05
Staphylococcus epidermidis	32(66.67%)	36(56.25%)	36(69.23%)	44(73.33%)	P>0.05
Streptococcus species	8(16.67%)	16(25.00%)	8(15.38%)	8(13.33%)	P>0.05
Proteus mirabilis	12(25.00%)	8(12.50%)	12(23.08%)	12(20.00%)	P>0.05
Enterococcus faecalis	4(8.33%)	4(6.25%)	4(7.69%)	8(13.33%)	P>0.05

Key: n = number of subjects, % = percentage.

Discussion:

In the present study, the total aerobic count (bacteria load) of petrol station staff ($58.72\pm2.28\times10^3$CFU/ml) compared favourably with the count of non-petrol station staff ($59.17\pm2.12\times10^3$CFU/ml) and did not differ significantly (P>0.05). This suggested that inhalation of petrol fumes may not have any effect on the bacteria that colonize the nose. The organisms may have the ability to survive and proliferate in the presence of low concentration of petrol fumes.

Staphylococcus aureus carrier rate did not differ so much for petrol station staff and non-petrol station staff, 32.14% and 28.57% respectively. This agreed with the work of Aikpitanyi-Iduitua *et al.* (2004) and falls within the range reported for healthy population (Stageman *et al.*, 1994; Osuide *et al.*, 1996). This suggests that working in the petrol station had no effect on the nasal *Staphylococcus aureus* carrier rates. Similar findings were observed for *Staphylococcus epidermidis.*

Also, there were favourable comparison between the Staphylococci carrier rates in male and female for both petrol station staff and non-petrol station staff. This corresponded with the report of Chigbu and Ezereonye (2003) that sex is not a notable factor in carriage and there is no activity or behavior peculiar to either of the sexes which may predispose them to Staphylococci infection.

In the present study, the carrier rate of *Streptococcus* species in petrol station (21.43%) staff and non-petrol station staff (14.43%) showed a slight variation. This may be attributed to personal hygiene and the fact that some of the workers may be carrier considering the occupational exposure which predisposes them to inhalation of fumes that affects the activities of the normal flora. Also, it may be due to the fact that as vehicles drive into the petrol station, dust with its load of bacteria is raised, which may settle in the nostril when inhaled. However, the variations observed in this study do not differ significantly (P>0.05).

In the present study the carrier rates of the pathogens, *Proteus mirabilis* and *Enterococcus faecalis* compared favourably in petrol station staff and non-petrol station staff. These organisms were isolated in lesser amount which agreed with the work of Miguel *et al.* (2002) who reported that these organisms rarely colonize the nares and as opportunistic pathogens, they are in constant check with the activities of the normal flora of the nose and can only cause infection if the activities of the normal flora are disrupted.

The distribution of the isolates among sex do not show any significant difference (P>0.05) in the present study for both petrol station staff and non-petrol station staff. This corresponded with the reports of Chigbu and Ezereonye (2003) that sex is not a notable factor in carriage and there is no activity or behavior peculiar to either of the sexes which may predispose them to the colonization by the organisms.

Conclusion:

The organisms encountered in the present study were opportunistic pathogens. The presence of these organisms in the nostril of petrol station staff if of public health importance because previous studies have shown that prolonged exposure to petrol can lead to decrease in host's immunity and resistance to infection (Cook, 1989; Miller and Chang, 2003). There is the risk of opportunistic infections with nose being the primary source. Therefore, in conclusion, it can be asserted from the results obtained in the present study that low concentration of petrol fumes has no significant effect on the bacteria colonization of the nose. However, petrol workers are advised to avoid direct and prolonged exposure or contact of the nostril to these volatile compounds and always use nose protector, air filter or nose mask in their dealings with petrol and other volatile compounds.

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