Prevalence and Antibiogram of Bacteria Associated with Mouthpiece of Shisha Equipment in Port Harcourt Metropolis

ABSTRACT

Shisha smoke is becoming more prominent among adolescent which is known to be carcinogenic posing a serious threat to public health as well as the risk of pathogenic bacteria associated with the mouthpiece. This research is carried out to determine the prevalence and antibiogram of bacteria associated with mouthpiece of shisha equipment in Port Harcourt metropolis. A total of twenty (20) mouthpiece of shisha equipment were swab using sterile swab sticks and samples subjected to standard microbiological technique as well as standard plate count, culturing, identification and antibiotic susceptibility pattern using Kirby Bauer Disk diffusion method. The total heterotrophic bacteria count ranged between $2.0\pm1.5 \times10^3$ to $6.5\pm5.3 \times10^3$ CFU/ml) Borokiri township and Ogunabali respectively. The total *Staphylococcal* count ranged from $1.6\pm0.5 \times10^2$ CFU/ml to $3.3\pm0.3 \times10^2$ CFU/ml in Ogunabali township and Borokiri sandfield respectively. A total of fourteen (14) bacteria isolates were identified belonging to the following genera; *Staphylococcus* spp, *Klebleslla* spp, *Bacillus* spp, *Pseudomonas* spp, *Micrococcus* spp and *Lactobacillus* spp. The prevalence of the bacteria indicated that *Staphylococcus* had the highest occurrence (28.57%), followed by *Pseudomonas* spp (7.07%), having the least prevalence across the locations. The Antimicrobial sensitivity testing results shows that *Staphylococcus* spp *Bacillus* spp and *Micrococcus* spp was more susceptible to Erythromycin, Gentamicin and Ofloxacin (100%) and resistant to Augmentin, Cefuroxime, Cloxacillin, Cefuroxime and Ceftazidime (100%). *Lactobacillus* spp, *Pseudomonas* spp and *Klebsiella* spp were more susceptible to Ciprofloxacin and Ofloxacin (100%) and resistant to Gentamicin, Nitrofurantoin, Ceftazidime, Cefuroxime, Augmentin and Cefixime (100%). These bacteria isolated are mostly pathogenic and may result in an increase in health issues as a result of non-hygienic protocol used during using mouth to mouth smoking with the shisha equipment. Medical personnel should enlighten the public especially the adolescent about the risks involved in smoking shisha.

Keywords: Mouthpiece, shisha equipment, Bacteria, Prevalence, Antibiogram, Port Harcourt.

Introduction

Shisha also known as waterpipe, narghile, argileh, hookan, hubble-bubble, goza, borry, qaylan, chica, and mada'a is a tobacco pipe with a long yet flexible tube that draws the smoke through water contained in a bowl. Even though hookah use in Nigeria is a recent trend, it has existed for a millennium. Emerging in the North Western provinces of India, spreading to Iran, the Arab world, and Turkey and now gaining popularity around the world (Smith-Simone *et al.,* 2008).

Shisha became very popular amongst youth when it was first introduced, gradually it found its way to people's houses, parties, functions and events. Cafes and restaurants have gathered a lot of acknowledgement by adding Shisha in variety of flavours in their menu card (Gatrad *et al.*, 2007). By seeing this acknowledgement almost all cafes are serving Shisha in Nigeria and some studies says that Shisha smoking is safer than tobacco cigarette smoking but medical professionals says that all kinds on smoking is bad for health, therefore Shisha stands at same level as is the cigarette regarding health issues (Cobb *et al.*, 2012). Each Shisha session typically

lasts for more than 40 minutes, and consists of 40 to 150 drags that each consists of 0.15 to 0.50 litres of smoke (Chaouachi, 2011). Hour long Shisha smoking is equivalent to 100-200 cigarettes; in a 45-minute smoking session a typical smoker would inhale 1.7 times the nicotine of a single cigarette.

A survey has estimated that almost 600% children have experienced this form of tobacco, as young as seven years old. Its smoke also contains hundreds of potentially dangerous substances including carbon monoxide, charcoal, nicotine, arsenic, cobalt, chromium and lead causing disorders including lung and bladder cancers, impaired pulmonary functions, coronary heart disease, infertility, tobacco dependence (Smith-Simone *et al*., 2008).

The water in Shisha which is assumed to be used to filter microorganisms is not that efficient to remove all kinds of microorganisms (Chaouachi, 2011). Humans are coming down with certain illnesses as a result of regular consumption of Shisha and lack of exchange of the water as well as mouth to mouth smoking of the shisha introduce pathogenic microorganism onto the mouthpiece of the shisha equipment which in turns spread to another humans during smoking with same mouthpiece (Chaouachi, 2011).

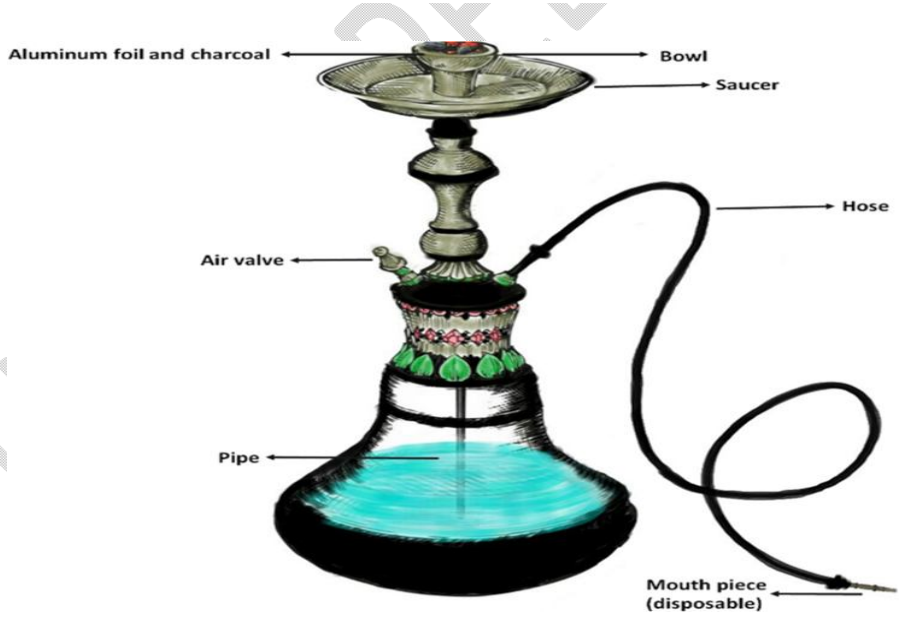

Fig. 1. A typical Shisha apparatus (Gatrad *et al.*, 2007)

Hence, this research is carried out to determine the prevalence and antibiogram of bacteria associated with mouthpiece of shisha equipment in Port Harcourt metropolis.

MATERIAL AND METHOD

Description of study Area

The study was carried out in four (4) different Location in Rivers State viz; Rivers State University, Borokiri sandfield, Ogbunabali township and Diobu-Mile 1 all within Port Harcourt Metropolis where shisha smoking activities are high.

Sample Collection

A total of twenty (20) piecing equipment were swab using sterile swab sticks from the four (4) different locations under aseptic condition in Port Harcourt Rivers State, and transported to the Department of Microbiology Laboratory Rivets State University for further bacteriological analyses.

Microbiological Analysis

Bacteria Enumeration

The enumeration of the total heterotrophic bacteria was carried out using nutrient agar while the total Staphylococcal count were performed on Mannitol salt agar. The stock analytical unit was done by moistening the swab stick with normal saline and swabbed over the surface of the mouthpiece of the shisha equipment and dipped into the 2ml of normal saline separately to make 10^1 dilutions for enumeration, isolation and identification. Two-fold serial dilution was performed subsequently by pipetting 1ml of the samples into 2ml of sterile normal saline up to four (4) dilutions. About 0.1 aliquot of the appropriate dilutions (was inoculated in duplicates onto already prepared sterile plates of nutrient agar, Mannitol salt agar using the spread plate technique and incubated at 37^oC for 24hours after which the plates were counted and recorded. Representative colonies were described and sub-cultured onto nutrient agar plates and incubated at 37^oC for 24hours to obtain pure cultures (Taylor, 2008)

Preservation of pure culture

The pure cultures were stored in 10% (v/v) glycerol suspension at -4^{o}C as a cryo-preservative agent to prevent the damage of the pure cultures during drying for further analysis.

Isolation and Identification of the Bacterial Isolates

The bacterial isolates were isolated based on their colonial/morphological characteristics such as the size, margin, surface, colour, elevation, texture and transparency and Identification was carried out through conducting series of biochemical tests such as Oxidase, Catalase, Coagulase, Citrate Utilization, Methyl red, Indole, Voges Proskauer and sugar fermentation tests to confirm the identity of the test organisms (Cheesbrough, 2005)

Antibiotic Susceptibility Testing

The antimicrobial susceptibility profiles of the bacterial isolates to conventional antibiotics were determined using the Kirby Bauer disk diffusion method on sterile Mueller-Hinton agar. Standardization of the bacterial isolates was carried out by adjusting to 0.5 McFarland turbidity standards containing x10^{8} cells. The swab is deepened into the bacterial suspension and streaked over the surface of the agar plates, rotating the agar plate 60^{o} each time to ensure even distribution of the inoculum. The plates were left to air dry for 3–5 min. Conventional antibiotics disk impregnated with Gentamicin (10µg), Cloxacillin (5µg), Erythromycin (5µg), Ofloxacin (5µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg) and Augmentin (30µg) were aseptically placed on the surface of the inoculated agar plate with sterile forceps. Each disk was pressed down to ensure full contact with the surface of the agar. The plates were then incubated for 24 hours at 33 to 35^{o}C in an inverted position. The zones of inhibition were measured in millimeter (mm) using a meter rule and compared to (CLSI, 2017)

Data Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used to analyze the data obtained from counts and the measurement of the zones of inhibition. Descriptive statistics was used to summarize all data obtained. T-test was carried out to test for significant difference ($p \leq 0.05$) in the bacterial counts from the different locations.

RESULTS

The result from table 1 showed that the total heterotrophic bacterial count was high mouthpiece shisha equipment from Ogunabali township (6.5±5.3 ×10^{3} CFU/ml) and Borokiri sandfield had

the least count ($2.0\pm1.5 \times10^3$ CFU/ml). The result of the total Staphylococcal count showed that Borokiri sandfield had the highest count ($3.3\pm0.3 \times10^2$ CFU/ml) and Ogunabali township had the least count ($1.6\pm0.5 \times10^2$ CFU/ml)

Table 1. Bacterial Population of the Mouthpiece of Shisha Equipment from the various Locations

Locations	THB /CFU/ml	TSC /CFU/ml
Rivers State University region	$5.3\pm0.25 \times10^3$	$3.0\pm0.5 \times10^2$
Borokiri Sandfield	$2.0\pm1.5 \times10^3$	$3.3\pm0.3 \times10^2$
Ogunabali Township	$6.5\pm5.3 \times10^3$	$1.6\pm0.5 \times10^2$
Diobu-Mile 1	$2.5\pm2.3 \times10^3$	$2.3\pm0.00 \times10^2$

Key: **THB**- Total Heterotrophic Bacterial Count; **TSC**- Total Staphylococcal count;

A total of fourteen (14) bacteria isolates were identified belonging to the following genera; *Staphylococcus* spp, *Klebleslla* spp, *Bacillus* spp, *Pseudomonas* spp, *Micrococcus* spp and *Lactobacillus* spp. The prevalence of the bacteria indicated that *Staphylococcus* had the highest occurrence (28.57%), followed by *Pseudomonas* spp (7.07%**),** having the least prevalence across the locations as showed in table 2**.**

Table 2. Prevalence of Bacterial Isolates Mouthpiece of Shisha Equipment from the various Locations

Isolate	A	B	C	D	TOTAL	Percentage (%)
***Staphylococcus* spp**	1	1	1	1	4	28.57
***Bacillus* spp**	1	0	0	1	2	14.29
***Lactobacillus* spp**	0	1		1	2	14.29
***Micrococcus* spp**	0	1	1	1	3	21.43
***Klebsialla* spp**	1	0	0	1	2	14.29
***Pseudomonas* spp**	0	1	0	0	1	7.07
Total	**3**	**4**	**2**	**5**	**14**	**100**

Key: A-Rivers University Back Area; **B**-Borokiri sandfield; **C**-Ogunabali township Area; **D**-Diobu-Mile 1

Table 3. Colonial/Morphological and Biochemical Characteristics of Bacterial Isolates from the various Locations

S/N	Isolate Code	Color	Elevation	Opacity	MMP	OX1	CAT	SH	CIT	STT	URS	MR	VP	IND	MOT	GLU	LAC	MAP	MAL	GLY	XYL	Suspected Organism
1	A1	Golden yellow	Raised	Opaque	GPC	-	+	-	+	-	-	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
2	A2	Creamy	Convex	Opaque	GNR	-	+	-	+	-	+	-	+	-	-	AG	AG	A	A	A	A	*Klebsiella* spp
3	A3	Cream	Raised	Flat	GPR	+	+	-	+	-	-	-	-	-	+	AG	N	A	A	A	A	*Bacillus* spp
4	B1	Golden yellow	Raised	Opaque	GPC	-	+	-	+	-	-	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
5	B2	Green	Umbonate	Opaque	GNR	+	+	+	+	-	+	-	-	-	-	A	N	A	A	A	A	*Pseudomonas* spp
6	B3	Light Yellow	Convex	Opaque	GPC	+	+	-	-	+	-	-	-	+	-	-	A	A	A	A	A	*Micrococcus* spp
7	B4	Milky	Raised	Opaque	GPR	-	+	+	-	-	-	-	+	-	-	A	A	N	A	A	N	*Lactobacillus* spp
8	C1	Greyish white	Raised	Translucent	GPC	+	+	-	-	+	-	-	+	-	-	A	A	A	A	A	A	*Micrococcus* spp
9	C2	Golden yellow	Raised	Flat	GPC	-	+	-	+	-	-	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
10	D1	Creamy	Convex	Creamy	GNR	-	+	-	+	-	+	-	+	-	-	AG	AG	A	A	A	A	*Klebsiella* spp
11	D2	Greyish white	Raised	Translucent	GPC	+	+	-	-	+	-	-	+	-	-	A	A	A	A	A	A	*Micrococcus* spp
12	D3	Milky	Raised	Opaque	GPR	-	-	+	+	-	-	-	+	+	-	A	A	N	A	A	N	*Lactobacillus* spp
13	D4	Golden yellow	Raised	Opaque	GPC	-	+	-	+	-	-	-	+	-	-	A	A	A	A	A	A	*Staphylococcus* spp
14	D5	Cream	Raised	Flat	GPR	+	+	-	-	+	-	-	-	-	+	AG	N	A	A	A	A	*Bacillus* spp.

Key: A-Rivers University Back gate; **B**-Borokiri sandfield; **C**-Ogunabali township; **D**-Diobu-Mile 1, MMP – Microscopic Morphology, GPC-Gram +ve Cocci, GLU – Glucose, MAN – Mannitol, MAL – Maltose, LAC – Lactose, XYL – Xylose, CAT – Catalase Test, OXI – Oxidase Test, MOT – Motility Tests, VP – Voges Proskaur Test, MR – Methyl Red, INO – Indole, CIT – Citrate, URS – Urea, SH – Starch Hydrolysis, STT – Salt Tolerance Test

The result of the susceptibility test as shown in Table 4-7 revealed that *Staphylococcus* spp *Bacillus* spp and *Micrococcus* spp was more susceptible to Erythromycin, Gentamicin and Ofloxacin (100%) and resistant to Augmentin, Cefuroxime, Cloxacillin, Cefuroxime and Ceftazidime (100%). *Lactobacillus* spp, *Pseudomonas* spp and *Klebsiella* spp were more susceptible to Ciprofloxacin and Ofloxacin (100%) and resistant to Gentamicin, Nitrofurantoin, Ceftazidime, Cefuroxime, Augmentin and Cefixime (100%).

Table 4. Susceptibility Pattern of *Staphylococcus* spp and *Bacillus* spp

Antibiotics	Conc.	*Staphylococcus* spp (n=4)			*Bacillus* spp (N=2)		
	µg	Susceptibility	Intermediate	Resistance	Susceptibility	Intermediate	Resistance
ERY	5	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)
CXC	5	0(0)	0(0)	4(100)	0(0)	0(0)	4(100)
OFL	5	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)
AUG	30	0(0)	0(0)	4(100)	0(0)	0(0)	4(100)
CAZ	30	0(0)	0(0)	4(100)	0(0)	0(0)	4(100)
CRX	30	0(0)	0(0)	4(100)	0(0)	0(0)	4(100)
GEN	10	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)
CTR	30	0(0)	0(0)	4(100)	0(0)	0(0)	4(100)

KEY: (GEN) Gentamicin, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacilin, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime

Table 5. Susceptibility Pattern of *Micrococcus* spp (N=3)

Antibiotics	Conc.	*Micrococcus* spp (n=3)		
	µg	Susceptibility	Intermediate	Resistance
ERY	5	4(100)	0(0)	0(0)
CXC	5	0(0)	0(0)	4(100)
OFL	5	4(100)	0(0)	0(0)
AUG	30	0(0)	0(0)	4(100)
CAZ	30	0(0)	0(0)	4(100)
CRX	30	0(0)	0(0)	4(100)
GEN	10	4(100)	0(0)	0(0)
CTR	30	0(0)	0(0)	4(100)

KEY: (GEN) Gentamicin, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime

Table 6. Susceptibility Pattern of *Klebsiella* spp and *Lactobacillus* spp

Antibiotics	Conc.	*Klebsiella* spp (n=2)			*Lactobacillus* spp (N=2)		
	µg	Susceptibility	Intermediate	Resistance	Susceptibility	Intermediate	Resistance
OFL	5	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
GEN	10	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)
NIT	300	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)
CRX	30	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)
CAZ	30	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)
CPR	5	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)
AUG	30	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)
CXM	5	0(0)	0(0)	2(100)	0(0)	0(0)	2(100)

KEY: GEN) Gentamycin, (CPR) Ciprofloxacin, (NIT) Nitrofurantoin, (CXM) Cefixime, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime.

Table 7. Susceptibility Pattern of *Pseudomonas* spp (N=1)

Antibiotics	Conc.	*Pseudomonas* spp (n=2)		
	µg	Susceptibility	Intermediate	Resistance
OFL	5	1(100)	0(0)	0(0)
GEN	10	0(0)	0(0)	1(100)
NIT	300	0(0)	0(0)	1(100)
CRX	30	0(0)	0(0)	1(100)
CAZ	30	0(0)	0(0)	1(100)
CPR	5	1(100)	0(0)	0(0)
AUG	30	0(0)	0(0)	1(100)
CXM	5	0(0)	0(0)	1(100)

KEY: GEN) Gentamycin, (CPR) Ciprofloxacin, (NIT) Nitrofurantoin, (CXM) Cefixime, (OFL) Ofloxacin, (AUG) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime.

DISCUSSION

It is estimated that thousands of Nigerians use shisha on a daily basis. Comparable to those levels, adults "current use-smoking water-pipe on at least 1 day within the past 30 days was 9.8% and "ever use-smoking water-pipe at any point in lifetime was 1.5% between 2009 and reaching levels of 12.3% and 3.3%, respectively, by 2012–2013, reflecting a gross increase within the Nigerian population

without considering the health implications ((Blank *et al.,* 2011). This increase in use could be attributed to the perception of fewer negative consequences of hookah (shish) smoking compared with cigarette smoking and the social norms regarding its acceptability among this population (Gatrad *et al.*, 2007). With tobacco being the main source of smoke in both shisha and cigarettes, shisha users are exposed to many of the same toxic compounds/by-products as cigarette users but at dramatically higher levels, which might in fact produce worsened health effects in users (Bou *et al.*, 2014). There was a high bacterial load from the mouthpiece shisha equipment from the various location probably because the person carrying out piercing do not wash his or her hands with a germicidal soap nor wear disposable gloves or use disposable or sterilized tools and the use old mouthpiece during smoking. Inappropriate hygiene increases the possibility of bacterial infections. Theoretically, sharing the mouthpiece during shisha group smoking can be a probable source of transmission of pathogens such as viruses, bacteria, and fungi. For instance, a study reported a potential risk for transmission of communicable diseases such as hepatitis C and erysipelas caused by *Staphylococcus aureus* when sharing the mouthpiece between users with bleeding gum (Gatrad, *et al* 2007). The presence of this pathogenic microorganism such as *Staphylococcus*, *Pseudomonas*, *Bacillus* that colonies the mouth piece of the shisha equipment can cause severe diseases to the user causing a great threat to the public and high prevalence of *Staphylococcus* could be due several sanitary factors such as; poor cleaning and hand hygiene, poor quality of raw materials and cross-contamination as well as the unclean water in the shisha equipment. *Staphylococcus* spp, *Bacillus* spp, *Lactobacillus, Klebsiella, Micrococcus* spp, *Pseudomonas* spp and where highly susceptible to the gentamicin, ofloxacin and ciprofloxacin. The drug ofloxacin and ciprofloxacin interferes with nucleic acid synthesis during DNA replication by inhibiting either DNA gyrase or topoisomerase IV (Akani *et al.*, 2021). Gentamicin belonging to aminoglycosides group is not surprising because it is known to be effective against most Gram negative bacteria by binding to their ribosomes and inhibiting protein synthesis as described by Vakulenko and Mobashery, (2003) and they were resistance to the penicillin class of antibiotic such as ceftazidime, cefuroxime could be explained by uncontrolled use of antibiotics in the treatment of infections and toxic chemicals in the shisha and the availability of these drugs non-restrictively in this areas which enables self-prescription and presence of beta lactamases enzyme possessed by this organisms as well as acquisition of resistant genes.

Conclusion and Recommendation

The research shows that mouthpiece of shisha equipment harbors many pathogenic bacteria and could endanger the individual and leads to diseases and infections that will affect the individual and also

become the tobacco in it can become carcinogenic. Personal hygiene should be encouraged to reduce the presence of pathogenic bacteria from habiting the mouth piece equipment and misuse of drugs should be discouraged to reduce the rate of antimicrobial resistance. The water in the shisha equipment should be changed regularly and government should enact laws to put labels on the shisha equipment, that smokers are liable to die young as done with cigarette.

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