

Prevalence of bacterial infection among narghile smokers complaining of respiratory problems in Kirkuk city, Iraq

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ABSTRACT

Respiratory infections by different bacteria that are raised from smoking narghile and electronic cigarettes are rising steadily. This study aims to determine the relation of smoking narghiles and electronic cigarettes with respiratory infection caused by bacterial infection among different age groups in Kirkuk city. Sputum samples were taken from 130 patients with typical signs and symptoms of respiratory infection who were narghile and electronic cigarette smokers. Bacterial isolation and identification, as well as, susceptibility test was done. Among 130 sputum samples, 79 (60.76%) gave positive bacterial growth. The dominant age group that smokes narghile and electronic cigarettes was (26-35). The results revealed that *Streptococcus pyogenes* was the most frequent bacteria associated with smoking narghiles and electronic cigarettes which constituted 24.05 % of bacterial isolates. followed by *Streptococcus pneumoniae* (16.45%) *Staphylococcus aureus* (13.92%), *Staphylococcus epidermidis* (8.86%), *Klebsiella pneumoniae* (11.39%), *Pseudomonas aeruginosa* (17.72%) and *Escherichia coli* (7.59%). Antibiotic susceptibility test revealed that most isolates were resistant to more than two antibiotics. Also, these isolates showed remarkable resistance to erythromycin especially *Pseudomonas aeruginosa* (85.7%). On the other hand, all Gram-positive isolates showed high sensitivity to vancomycin (100%), while *E. coli* showed resistance to vancomycin and amoxicillin (100%) and (83.3%) respectively. It is concluded that narghile and e-cigs smokers at age (15-25 years) are at risk of bacterial respiratory infection. More isolated bacteria are multi-resistant to antibiotics. It is recommended to know the catastrophic impact of smoking narghiles, electronic cigarette on the lungs and knows their association in increasing susceptibility to bacterial infection.

Keywords: Narghile, Electronic cigarette, Bacteria, Antibiotics, Respiratory infection

Introduction

Narghile, which is also known as water pipe and shisha, is worldwide distributed especially among youth. There is a remarkable raised risk of pulmonary infections due to narghile use as different parts of the instrument can play a role as a reservoir for pathogens. It has been found that culturing from

the water storage and oral pieces from different narghile cafes carry a high percentage of bacterial contamination with pathogenic bacteria [1-5].

Electronic cigarettes (e-cigs) are common among people as a better replacement for the smoking of tobacco and recently their use became very common [6]. Recent studies prove that their association with acute pulmonary disease is due to vaping nicotine-containing electronic cigarettes [7]. Bacteria, especially *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was implicated in the occurrence of chronic pulmonary disease related to smoking, through direct bacterial infection and even though inflammation [8]. Different studies showed that bacterial infections are correlated with lung damage and diminish lung function by vaping e-cigs [9].

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This study aimed to determine the correlation of narghile and e-cigs smoking with respiratory infection caused by pathogenic bacteria in Kirkuk city. Also, the susceptibility pattern of bacterial isolates to the most common antibiotics was studied.

Materials and Methods

One hundred and thirty sputum samples were taken from patients complaining of respiratory infections who were attended to at Azadi Teaching Hospital at Kirkuk City during a period between the 1st of February 2019 to the end of July 2019. All patients were male their ages ranged between 15 to 55 years. Every patient was instructed to rinse their mouth with water to remove excess oral flora then asked to cough deeply and spit sputum in a sterile plastic container and immediately transported to the laboratory under the aseptic condition for bacterial culturing and susceptibility tests.

Bacteriological study

The sputum samples were cultured on MacConkey and Nutrient agars then incubated at 37°C for 24 hours. The growing colonies were further cultured on other selective media as Eosin methylene blue, Mannitol salt agar, and Pseudomonas Agar Base +CN (Cetrimide, Nalidixic acid) a selective medium used for isolation of *Pseudomonas aeruginosa*. Gram staining and biochemical tests Bergey's manual of determinative bacteriology was done for the colonies of pure isolates for identification [10]. Then the results were confirmed by RapID™ONE System remel (USA) for identification of Enterobacteriaceae, Api Staph. and Api Strep. Systems (BioMérieux, France).

Antibiotic susceptibility test

The disc diffusion method was used to clarify the susceptibility and sensitivity of bacterial isolates against common antibiotics used in Iraq. The antibiotics included amoxicillin (AMC) 30 mcg, amikacin (AK) 30 mcg, azithromycin (AZM) 15mcg, ciprofloxacin (CIP) 5mcg, chloramphenicol (C) 30 mcg, erythromycin (E) 15 mcg, cefotaxime (CTX) 30 mcg, tobramycin (TOB)10 mcg, vancomycin (VA) 30 mcg and gentamicin (CN) 10 mcg (Carolina Biological Supply Company USA). Inoculum preparation was done by transferring a single colony into 5ml nutrient broth and then incubated at 37°C for 24hr, and then the turbidity was adjusted to 0.5 McFarland turbidity standard solutions. The inoculum spread eventually with a sterile swab on the surface of Muller- Hinton agar. The standard antibiotic discs were placed at determined points in the same Petri dishes and left a while before incubation at 37°C for 24hr. Zones of inhibition diameters are measured and interpreted according to the Clinical and Laboratory Standards Institute [11].

Results and Discussion

The results showed that the high percentage of smokers 47 (36.15%) was among the age group (26-35 years). A study that was done by Salloum and his coworkers [12] found that waterpipe tobacco smoking is more prevalent among males (13.4%) and the highest (28.4%) smokers were among the age group (18-24 years). These differences may be due to cultural differences between countries. Out of 130 sputum samples taken from narghile and e-cigs smokers, 79 (60.7%) gave positive bacterial growth. The highest positive growth (81.81%) was revealed among the age group (15-25 years), while the lowest positive growth (42.85%) was revealed among the age group (46-55 years) (Table 1).

Table 1. Distribution of positive bacterial growth among narghile and e-cigs smokers according to age groups.

Age groups (Year)	Total No.	%	Number of infected samples	%
15-25	33	25.38	27	81.81
26-35	47	36.15	23	48.93
36-45	29	22.30	20	68.96
46-55	21	16.15	9	42.85
Total	130	100	79	60.76

The majority of bacterial isolates were Gram-positive bacteria 50 (63.29%), while Gram-negative bacteria comprised 29 (32.91%) (Figure 1). A similar finding was published by Majed *et al.* [13] who cultured swab samples from water pipe hoses.

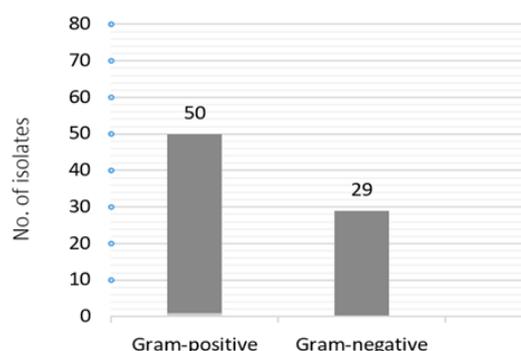


Figure 1. Bacterial isolates from sputum sample according to Gram staining test

Out of 130 samples, 79(60.76%) gave positive bacterial growth. The Gram-positive isolated bacteria identified as *Streptococcus pyogenes* 19 (24.05%), *S. pneumonia* 13(16.45%), *Staphylococcus aureus* 11(13.92%), *S. epidermidis* 7(8.86%). The Gram-negative bacteria were *Pseudomonas aeruginosa* 14(17.72%), *Klebsiella pneumoniae* 9(11.39%), and *Escherichia coli* 6(7.59%). Among Gram-positive bacteria, *S. pyogenes* was the predominant isolate 19(24.05%), and *P. aeruginosa* was among gram negatives (Table 2).

Table 2. Frequency and percentages of bacteria isolated from patients with respiratory infection smoking narghile and e-cigs.

No.	Bacterial isolates	Number of isolates	Percentage %
1	<i>Streptococcus pyogenes</i>	19	24.05
2	<i>Streptococcus pneumonia</i>	13	16.45
3	<i>Staphylococcus aureus</i>	11	13.92
4	<i>Staphylococcus epidermidis</i>	7	8.86
5	<i>Pseudomonas aeruginosa</i>	14	17.72
6	<i>Klebsiella pneumonia</i>	9	11.39
7	<i>Escherichia coli</i>	6	7.59
Total		79	100

Smoking plays role in the development of lung diseases such as bronchitis and pneumonia caused by bacteria. Most of these bacterial isolates are part of the lung microbiota in healthy individuals but under particular conditions like electronic cigarette vapor that cause lung injury lead to respiratory infections. Electronic cigarette use increases susceptibility to bacterial infection especially by Gram-negative bacteria [14]. In a study, cigarette smoking is considered to be the cause of changes in the respiratory tract structure and decreases the immune response. Cigarette smoking is considered a substantial risk factor for infection with important bacteria and viruses. Smokers provoke a two to four-fold increased invasive risk of

pneumococcal disease [15]. Alexander *et al.* [16] found that e-cigs vapor raises staphylococcal virulence and damages innate immune function.

A finding was published by Nuorti *et al.* [17] who found that the bacterial respiratory infection increase among adult smokers including *Streptococcus pneumonia*, also, Gilpin *et al.* [18] reported that smoking-related chronic lung infections proved due to many bacteria particularly *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* through both direct infection and bacteria-mediated inflammation. He also proved that the bacteria normally found in the lung become more harmful by causing inflammation when they have been exposed to e-cigarette vapor.

In the present study, most of the bacterial isolates showed resistance to more than three antibiotics, it is assumed to be multidrug-resistant (**Table 3**). High rate resistance to erythromycin with moderate resistance rate to tobramycin and less resistance to vancomycin was observed. Most isolates were resistant to amoxicillin especially *Pseudomonas aeruginosa* (78.5%). *S. pyogenes* showed remarkable resistance to amikacin and tobramycin (84.5%). Most isolates were resistant to vancomycin especially *E. coli*, *P. aeruginosa*, *K. pneumoniae* (100%). This is reasonable because it is a narrow-spectrum antibiotic and has no effect on gram-negative. All gram-positive isolates were found to be highly sensitive 100% to vancomycin.

Table 3. Illustrates antibiotic susceptibility pattern of all tested isolates

Antibiotics	Number and percentage of resistant Bacteria that isolated from cases													
	<i>S.pyogenes</i>		<i>S.pneumonia</i>		<i>S.aureus</i>		<i>S.epidermidis</i>		<i>P.aeruginosa</i>		<i>K.pneumoniae</i>		<i>E.coli</i>	
	19		13		11		7		14		9		6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AMC	6	31.5	1	7.6	7	63.3	4	57.1	11	78.5	5	55.5	5	83.3
AK	16	84.2	11	84.6	4	36.6	3	42.8	2	14.2	2	22.2	1	16.6
AZM	11	57.8	10	76.9	2	18.1	5	71.4	3	21.4	4	44.4	0	0
CIP	6	31.5	3	23	1	9	3	42.8	2	14.2	1	11.1	2	33.3
CTX	10	52.6	2	15.3	2	18.1	3	42.8	10	71.4	4	44.4	0	0
C	9	47.3	2	15.3	2	18.1	2	28.5	7	50	3	33.3	1	16.6
CN	15	78.9	9	69.2	2	18.1	4	57.1	3	21.4	4	44.4	0	0
E	10	52.6	9	69.2	3	27.2	5	71.4	12	85.7	6	66.6	3	50
TOB	16	84.2	11	84.6	3	27.2	4	57.1	3	21.4	5	55.6	2	33.3
VA	0	0	0	0	0	0	0	0	14	100	9	100	6	100

AMC: amoxicillin AK: amikacin, AZM: azithromycin CIP: ciprofloxacin C: chloramphenicol E: erythromycin CTX: cefotaxime TOB: tobramycin VA: vancomycin CN: gentamicin

Conclusion

The awareness about the most common infectious microorganisms in patients having signs of respiratory infection and smoking narghile and e-cigarette as an alternative to tobacco smoking, infections by gram-positive was more than gram-negative. Narghile and e-cigs smokers at age (15-25 years) are at risk of bacterial respiratory infection. More isolated bacteria are multi-resistant to antibiotics.

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