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Original article

Staphylococcus aureus nasal colonization among Vietnamese adults: prevalence, risk factors and antibiotic susceptibility profile

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Abstract: *Staphylococcus aureus (S. aureus)* has long been recognized as an important human pathogen causing many severe diseases. It is also a part of human normal flora with its ecological niche in the human anterior nares. This study focused on screening *S. aureus* nasal carriage in community and its relationship to human physiological and pathological factors which have not been studied in Vietnam previously. Two hundred and five volunteers in Ho Chi Minh City from 18 to 35 and over 59 years old both male and female participated in the study. Result showed that the prevalence of *S. aureus* nasal carriage in southern Vietnamese community was relatively low, only 11.2% (23/205), much lower than that in other international reports on human *S. aureus*. In addition, nasal carriage of the older age group (> 59 years old, 13.7%) was higher than that of younger age (18-35 years old, 10.4%). Other potential risk factors such as gender, career, height, weight, history of antibiotic usage, daily nasal wash, use of nasal medication sprays, acne problems, smoking and nasal problems showed no significant impact on *S. aureus* carriage. The obtained *S. aureus* nasal isolates were all sensitive to vancomycin. Lincomycin and tetracycline had low resistance rate with 4.3 % and 17.4 %, respectively. However, the isolates showed particularly high rate of multidrug resistance (54.2%) In summary, our data provided researchers an overview on *S. aureus* nasal carriage and antibiotic susceptibility profile of the community- isolated *S. aureus* in Vietnam. This would serve as valuable information on assessing risk of community-acquired *S. aureus* infections.

Keywords: Antibiotic resistance, nasal colonization, risk factors, *Staphylococcus aureus*, Vietnamese.

1. INTRODUCTION

Staphylococcus aureus (S. aureus) is widespread colonizer of human body surface of which the anterior nostrils is the most frequent carriage site [1]. Studies have shown that *Staphylococcal* nasal carriage is a potential risk of community- acquired (CA) *Staphylococcal* infections which are a danger of public health [2-5]. In addition, the global increasing resistance of *S. aureus* to various antibiotics complicates treatment for its infections [6], among those methicillin-resistant *S. aureus* (MRSA) infections are always of most serious and difficult- to- treat ones [7-9]. In this study, we aimed to figure out the prevalence of *S. aureus* nasal carriage in southern Vietnamese community, associating the potential human risk factors with *S. aureus* nasal carriage and revealing antibiotic susceptibility of the nasal isolates which could later affect the outcome of CA-*Staphylococcal* infections treatment.

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2. MATERIALS AND METHOD

2.1. Study population

From September to December 2013, volunteers from different places in Ho Chi Minh City, Vietnam were recruited for the study following 2 groups of age: Group 1, 18 – 35 years old and Group 2, over 59 years old, with the proportion of three (Group 1) to one (Group 2) following the age ratio of Vietnamese population (General Statistics Office of Vietnam, 2011). Persons who were having fever at the time of sampling or hospitalized in the previous month were excluded.

2.2. Sample and data collection

Sample was collected from nasal cavity by rotating a sterile swab in the nares of each participant. Amies transport medium with charcoal (TITAN MEDIA, India) was used to carry samples to the laboratory for culture and identification. At the same time, the information on age, gender, career, height, weight, history of antibiotic usage in the past 2

months, daily nasal wash with water, use of nasal medication sprays, acne problems, smoking, nasal problems (such as asthma or sinusitis) and history of S. aureus infection of every participant was also collected.

2.3. Culture and identification of *S. aureus*

Samples were cultured on mannitol salt agar (MSA; HiMedia, India). All colonies surrounded by yellow zones on MSA after 24 hours of incubation at 37°C were selected for identification using Staphylase test kit (Oxoid, UK).

2.4. In-vitro antibiotic susceptibility testing

Sensitivity test was performed followed the Kirby-Bauer disc diffusion method. Inoculum used for the test was prepared from 1-3 colonies picked from culture plate and suspended in 5mL sterilized Mueller Hinton Broth (MHB; HiMedia, India) and adjusted for proper cell density using optical density at 600 nm. Antibiotic discs (Nam Khoa Biotek, Vietnam) used in this test included: ampicillin (10µg), cephalexin (30µg), meropenem (10µg), kanamycin (30µg), erythromycin (15 µg), clindamycin (2µg), tetracycline (10µg), vancomycin (30µg), ciprofloxacin (5µg) and linezolid (30µg). Diameter of inhibition zones was recorded in millimeter after 24 hours of incubation at 37oC. The antibiotic susceptibility was interpreted via CLSI guidelines [10].

2.4. Data analysis

Statistical Package for the Social Sciences (SSPS) for Windows (Version 16.0) software was used to statistically analyze the association between risk factors and *S. aureus* nasal carriage. The results were presented with 95% confidence interval (CI) and corresponding p value. The level of significance was set at 0.05 using the two-tailed method.

3. RESULTS AND DISCUSSION

3.1. The prevalence of *S. aureus* nasal carriage

In total, there were 205 volunteers joined this study. These comprised 96 males (46.8%) and 109 females (53.2%) with ages ranging from 18 to 94 years of which 154 were in Group 1 (18-35 years old) and 51 were in Group 2 (over 59 years old).

Percentage of nasal carriers in Group 1 is 10.4% (16/154) while Group 2 is 13.7% (7/51). Overall, nasal carriage of *S. aureus* in this study population was 11.2% (23/205) which is lower than a study in urban and rural northern Vietnam (15.8%, 161/1016 for nasal carriage and 29.7% for nasopharyngeal carriage, 302/1016) which was carried also with young people (35% of the cohort < 20 years old) who generally have markedly higher rate of nasal carriage [11]. This percentage is also slightly lower than a recent study carried out on 838 patients at an ICU in southern Vietnam (13.1%) [12]. In addition, in our study carriage prevalence was similar between males and females (11.5% and 11.0%) which were in agreement with previous study [11]. It seems that even though this study has relatively small sample size, the result still reflected quite well the status of big population. The rate of *S. aureus* nasal carriage in relationship with some risk factors is shown in Appendix A.

There was not any significant effect of the risk factors to the rate of *S. aureus* carriage in both groups based on data analysis (data not showed) even though differences in the percentages of *S. aureus* carriage were observed in each group of age (Appendix A).

Potential risk factors for nasal *S. aureus* carriage have been studied but none of them were significant. It is probably due to the fact that our sample size was too small for each factor to provide a significant value. For example, with the factor of the history of using antibiotic during last 2 months, it was observed that percentage of carriers in both groups was lower for antibiotic using people (7.7 % compared to 12.1 %) but still not statistically significant (analysis not shown). It has been shown that smokers were less likely to become *S. aureus* carrier than non-smokers [13] but in our study a similar pattern could only be found in group 1 but not in group 2 due to limited sample. Other factors such as cleaning nose with water or nasal spray and health conditions such as obesity or sinusitis and asthma problems which have been shown to have relationship with *S. aureus* colonization did not achieve significant level in our study [14].

3.2. Antibiotic susceptibility profile of *S. aureus* nasal isolates

In this study, resistant rate of *S. aureus* isolates were found to be 95.7% for ampicillin, 34.8% for cephalexin, erythromycin, kanamycin, and clindamycin, 47.8% for meropenem, 17.4% for tetracycline, 21.7% for ciprofloxacin and 4.3% for linezolid. Especially, vancomycin continued to be the treatment of choice for treating most MRSA infections with none of isolates (0%) resistant to this agent. The data was summarized in Figure 1. The prevalence of multi-drug resistance (resistant to at least 3 tested antibiotics) of *S. aureus* isolates was shown in Figure 2. There were thirteen isolates with resistance to at least 3 antibiotics of which two isolates were identified to be resistant to 8 antibiotics. Only one isolate was susceptible to all antimicrobial agents.

With the high resistance rate, ampicillin, meropenem and even cephalexin, clindamycin, erythromycin and kanamycin were not suggested for *S. aureus* infections treatment. It is different to recent study showing that first generation cephalosporins (cefazolin, cephalothin and cephalexin), clindamycin and erythromycin have important therapeutic roles in less serious *S. aureus* infections such as skin and soft tissue infections or in patients with penicillin hypersensitivity (urticaria, angioedema, bronchospasm or anaphylaxis) [15]. Besides, in our study, ciprofloxacin and tetracycline are two antibiotics to which *S. aureus* has low resistant rate (21.7% and 17.4%, respectively). Our results also suggested that linezolid and vancomycin remain important in the treatment of *S. aureus* infections because of high susceptible rate– 95.7% and 100%, respectively. It is in agreement with previous results showing that vancomycin has been considered the treatment of choice for infection due to *S. aureus* [16].

Multidrug resistance was frequently observed in this study with thirteen (54.2%) of all the isolates resistant to at least three antibiotics. Only one isolate (4.2%) was

fully susceptible to all the tested antimicrobial drugs. The multidrug-resistant *S. aureus* rate is comparable to what was reported in Nigeria (52.5%) [17] and lower than in other countries – 75% in Bangladesh [18] and over 94% in India [19]. However, compared to developed country, multidrug resistant *S. aureus* rate in this study is markedly higher – 32% in The USA and 24.6% in Europe [20-21].

4. CONCLUSION

In our study, some risk factors influenced *S. aureus* nasal carriage rate when analyzing them in different age groups but not in the whole population. Results also indicated that rate of *S. aureus* nasal colonization can be reduced via improving personal hygiene, such as performing hand washing frequently and effectively.

The study showed low rate of *S. aureus* nasal carriage among healthy Vietnamese but a high prevalence of multi-drug resistance in nasal *S. aureus* isolates. Resistance to beta-lactams and commonly prescribed antibiotics was dominant. Data suggested that it is important to control multi-drug resistance not only in healthcare systems but also in community to prevent the infections potentially caused by these multi-drug resistant isolates.

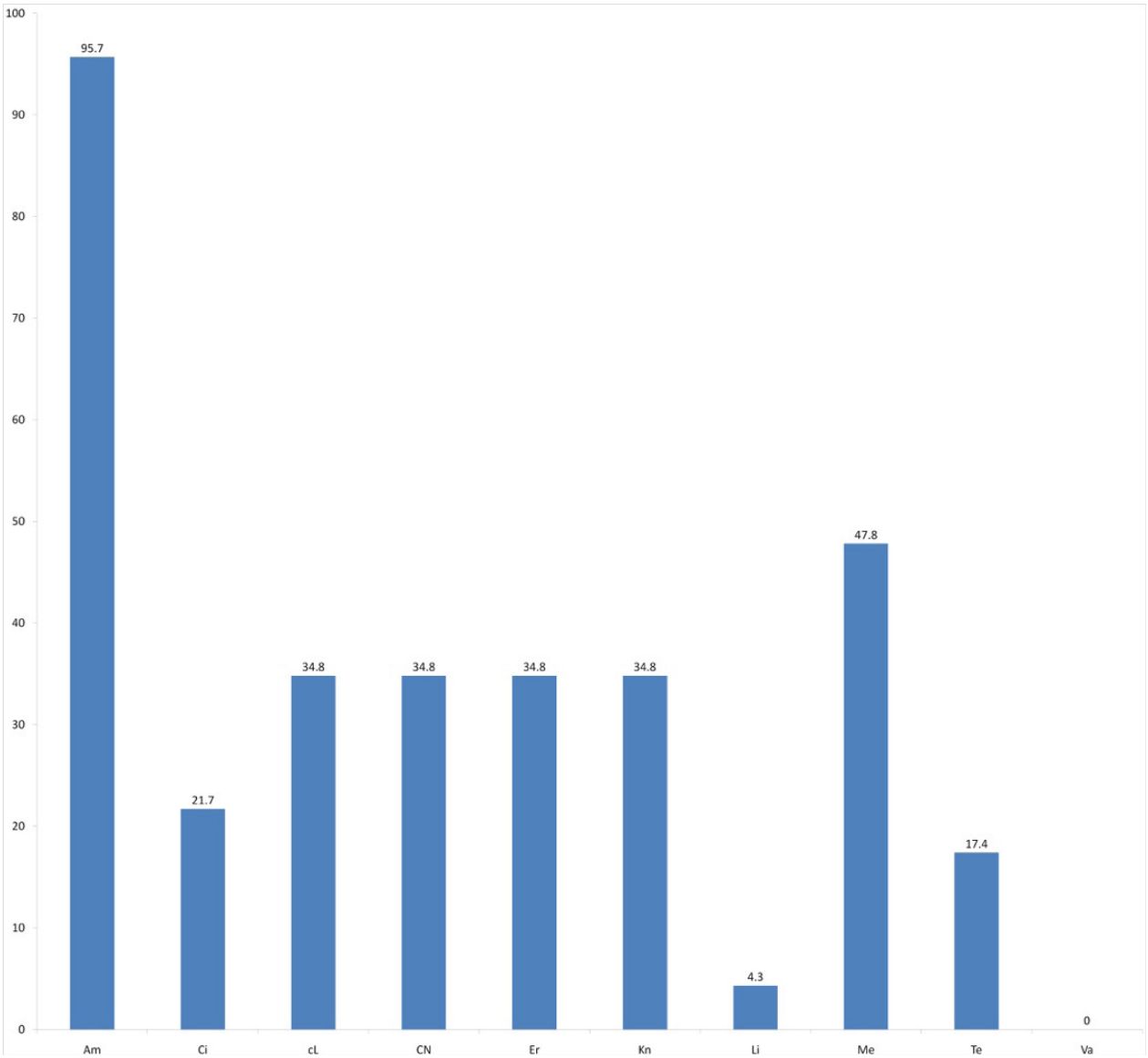

Figure 1. Antibiotic resistance rates of Staphylococcus aureus nasal isolates.
Am, ampicillin; Ci, ciprofloxacin; cL, clindamycin; CN, cephalexin; Er, erythromycin; Kn, kanamycin; Li, linezolid; Me, meropenem; Te, tetracycline; Va: vancomycin.

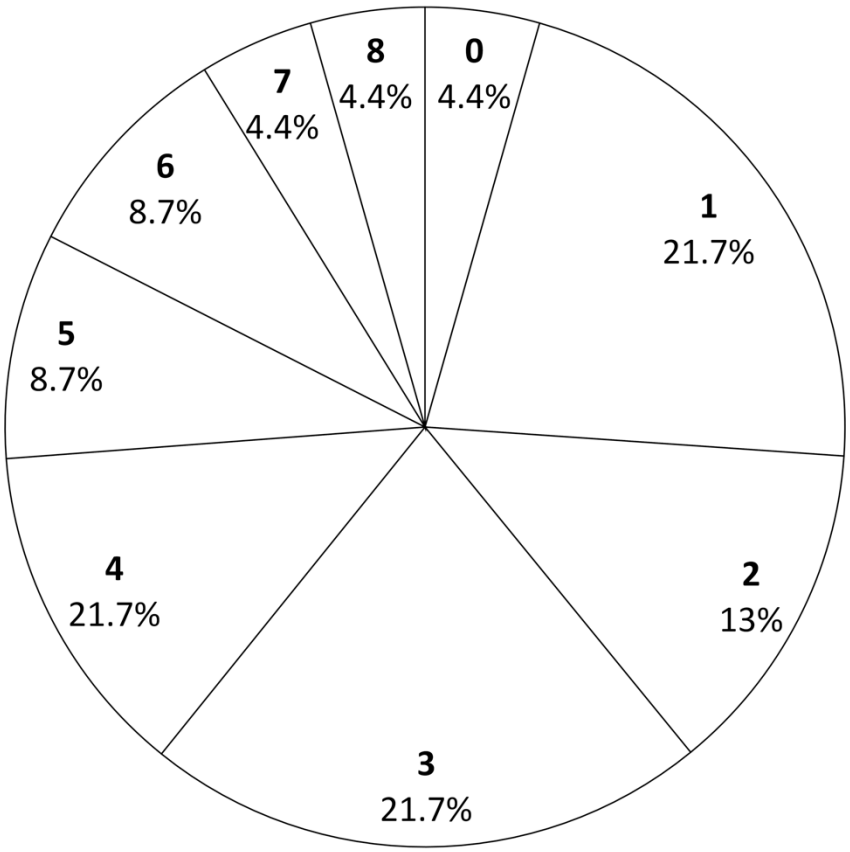

Figure 2. Prevalence of multi-drug resistance among S. aureus nasal isolates. Number of antibiotics to which resistance occurred: 0, 1, 2, 3, 4, 5, 6, 7, 8. MDR (Resistant to >= 3 different antibiotics) rate is found to be 69.6 %.

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Authors' contributions

PKN participated in data collection and identification of all sample. PTTH performed antibiotic susceptibility testing. NNT performed data interpretation and drafting of the manuscript. NTTH participated in all activities.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Figures

Figure 1. Antibiotic resistance rates of *Staphylococcus aureus nasal isolates*. Am, ampicillin; Ci, ciprofloxacin; cL, clindamycin; CN, cephalexin; Er, erythromycin; Kn, kanamycin; Li, linezolid; Me, meropenem; Te, tetracycline; Va: vancomycin.

Figure 2. Prevalence of multi-drug resistance among *S. aureus* nasal isolates. Number of antibiotics to which resistance occurred: 0, 1, 2, 3, 4, 5, 6, 7, 8. MDR (Resistant to >= 3 different antibiotics) rate is found to be 69.6 %.

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Appendix A: Factors associated with *Staphylococcus aureus* colonization

Factors	Group		Total participants (n)	Carriers of *S. aureus* (n')	Carriers of *S. aureus* (%)
Gender	1	• Male	74	7	9.5
		• Female	80	9	11.2
	2	• Male	22	4	18.2
		• Female	29	3	10.3
	Both	• Male	96	11	11.5
		• Female	109	12	11.0
Taking antibiotic during last 2 months	1	• Yes	31	3	9.7
		• No	123	13	10.6
	2	• Yes	8	0	0
		• No	43	7	16.3
	Both	• Yes	39	3	7.7
		• No	166	20	12.1
Smoking	1	• Yes	13	0	0
		• No	141	16	11.4
	2	• Yes	12	3	25.0
		• No	39	4	10.3
	Both	• Yes	25	3	12.0
		• No	180	20	11.1
Using nasal spray	1	• Yes	9	1	11.1
		• No	145	15	10.3
	2	• Yes	4	0	0
		• No	47	7	14.9
	Both	• Yes	13	1	7.7
		• No	192	22	11.5
Nasal wash (by water)	1	• Yes	37	5	13.5
		• No	117	11	9.4
	2	• Yes	10	0	0
		• No	41	7	17.1
	Both	• Yes	47	5	10.6
		• No	158	18	11.4
Acne	1	• Yes	24	2	8.3
		• No	130	14	10.8
	2	• Yes	0	0	n/a
		• No	51	7	13.7
	Both	• Yes	24	2	8.3
		• No	181	21	11.6
Nose conditions (Sinusitis, asthma)(†)	1	• Yes	11	4	36.4
		• No	143	12	8.4
	2	• Yes	8	0	0
		• No	43	7	16.3
	Both	• Yes	19	4	21.1
		• No	186	19	10.2

Factors		Group	Total participants (n)	Carriers of *S. aureus* (n')	Carriers of *S. aureus* (%)
Body Mass Index (BMI, kg/m^2)(‡)	**1**	• Underweight o (< 18.5 kg/m^2)	22	2	9.1
		• Normal range o (18.5 – 23.0 kg/m^2)	98	11	11.2
		• Overweight o (≥ 23.0 kg/m^2)	34	3	8.8
	2	• Underweight o (< 18.5 kg/m^2)	8	0	0
		• Normal range o (18.5 – 23.0 kg/m^2)	28	4	14.3
		• Overweight o (≥ 23.0 kg/m^2)	15	3	20.0
	Both	• Underweight o (< 18.5 kg/m^2)	30	2	6.7
		• Normal range o (18.5 – 23.0 kg/m^2)	126	15	11.9
		• Overweight o (≥ 23.0 kg/m^2)	49	6	12.2
		Total	**205**	**23**	**11.2**

(†) There was only one case suffering asthma.

(‡) BMI is calculated via weight (in kilograms) over height squared (in centimeters), applied for Asian people (WHO, 2004).

Appendix B: Data analysis using Chi-2 test

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Factors * Group	51	100.0%	0	0.0%	51	100.0%

Factors * Group Crosstabulation

			Group			Total
			Group 1	Group 2	Both	
Factors	Gender	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	Taking a.b	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	smoking	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	Using nasal	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	Nasal wash	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	Acne	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	nose	Count	2$_a$	2$_a$	2$_a$	6
		Expected Count	2.0	2.0	2.0	6.0
	BMI	Count	3$_a$	3$_a$	3$_a$	9
		Expected Count	3.0	3.0	3.0	9.0
Total		Count	17	17	17	51
Expected Count		17.0	17.0	17.0	51.0	

Each subscript letter denotes a subset of Group categories whose column proportions do not differ significantly from each other at the .05 level.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.000[a]	14	1.000
Likelihood Ratio	.000	14	1.000
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	51		

a. 24 cells (100.0%) have expected count less than 5. The minimum expected count is 2.00.

Risk Estimate

	Value
Odds Ratio for Factors (Gender / Taking a.b)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Chi-square test for Factors x Condition

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Factors * Condition	51	100.0%	0	0.0%	51	100.0%

Factors * Condition Crosstabulation

			Condition							Total
			yes	no	male	female	Low	Mid	High	
Factors	Gender	Count	0_a	0_a	3_b	3_b	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	Taking a.b	Count	3_a	3_a	0_a	0_a	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	smoking	Count	3_a	3_a	0_a	0_a	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	Using nasal	Count	3_a	3_a	0_a	0_a	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	Nasal wash	Count	3_a	3_a	0_a	0_a	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	Acne	Count	3_a	3_a	0_a	0_a	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	nose	Count	3_a	3_a	0_a	0_a	0_a	0_a	0_a	6
		Expected Count	2.1	2.1	.4	.4	.4	.4	.4	6.0
	BMI	Count	0_a	0_a	0_a	0_a	3_b	3_b	3_b	9
		Expected Count	3.2	3.2	.5	.5	.5	.5	.5	9.0
Total		Count	18	18	3	3	3	3	3	51
Expected Count			18.0	18.0	3.0	3.0	3.0	3.0	3.0	51.0

Each subscript letter denotes a subset of Condition categories whose column proportions do not differ significantly from each other at the .05 level.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	102.000[a]	42	.000
Likelihood Ratio	81.982	42	.000
Linear-by-Linear Association	6.392	1	.011
N of Valid Cases	51		

a. 56 cells (100.0%) have expected count less than 5. The minimum expected count is .35.

Risk Estimate

	Value
Odds Ratio for Factors (Gender / Taking a.b)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Appendix C: Data analysis using ANOVA

Gender

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Male	3	13.0333	4.56795	2.63731	1.6859	24.3807	9.46	18.18
Female	3	10.8667	.47163	.27229	9.6951	12.0383	10.34	11.25
Total	6	11.9500	3.13748	1.28087	8.6574	15.2426	9.46	18.18

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.042	1	7.042	.668	.460
Within Groups	42.177	4	10.544		
Total	49.219	5			

Acne

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	2	9.5500	1.72534	1.22000	-5.9516	25.0516	8.33	10.77
2.00	2	6.8650	9.70858	6.86500	-80.3631	94.0931	.00	13.73
3.00	2	9.9650	2.31224	1.63500	-10.8096	30.7396	8.33	11.60
Total	6	8.7933	4.77299	1.94857	3.7844	13.8023	.00	13.73

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.328	2	5.664	.166	.855
Within Groups	102.580	3	34.193		
Total	113.907	5			

BMI

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	9.7100	1.31465	.75901	6.4442	12.9758	8.82	11.22
2.00	3	11.4300	10.30217	5.94796	-14.1620	37.0220	.00	20.00
3.00	3	10.2700	3.12232	1.80267	2.5137	18.0263	6.67	12.24
Total	9	10.4700	5.47542	1.82514	6.2612	14.6788	.00	20.00

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.618	2	2.309	.059	.943
Within Groups	235.224	6	39.204		
Total	239.841	8			

Nasal wash

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	2	11.4550	2.90621	2.05500	-14.6563	37.5663	9.40	13.51
2.00	2	8.5350	12.07031	8.53500	-99.9125	116.9825	.00	17.07
3.00	2	11.0150	.53033	.37500	6.2502	15.7798	10.64	11.39
Total	6	10.3350	5.73295	2.34047	4.3186	16.3514	.00	17.07

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.914	2	4.957	.096	.911
Within Groups	154.420	3	51.473		
Total	164.333	5			

Nose condition

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	2	22.3750	19.77778	13.98500	-155.3213	200.0713	8.39	36.36
2.00	2	8.1400	11.51170	8.14000	-95.2885	111.5685	.00	16.28
3.00	2	15.6350	7.65797	5.41500	-53.1691	84.4391	10.22	21.05
Total	6	15.3833	12.53116	5.11582	2.2327	28.5340	.00	36.36

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	202.825	2	101.413	.522	.639
Within Groups	582.324	3	194.108		
Total	785.149	5			

Smorking

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	2	5.6750	8.02566	5.67500	-66.4327	77.7827	.00	11.35
2.00	2	17.6300	10.42275	7.37000	-76.0147	111.2747	10.26	25.00
3.00	2	11.5550	.62933	.44500	5.9007	17.2093	11.11	12.00
Total	6	11.6200	7.95457	3.24744	3.2722	19.9678	.00	25.00

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	142.935	2	71.467	1.236	.406
Within Groups	173.441	3	57.814		
Total	316.376	5			

Taking a.b

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	2	10.1250	.62933	.44500	4.4707	15.7793	9.68	10.57
2.00	2	8.1400	11.51170	8.14000	-95.2885	111.5685	.00	16.28
3.00	2	9.8700	3.08299	2.18000	-17.8295	37.5695	7.69	12.05
Total	6	9.3783	5.42375	2.21424	3.6865	15.0702	.00	16.28

ANOVA

Carrier

	Sum of Squa res	df	Mean Square	F	Sig.
Between Groups	4.665	2	2.333	.049	.953
Within Groups	142.420	3	47.473		
Total	147.085	5			

Using spray

Descriptives

Carrier

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	2	10.6750	.61518	.43500	5.1478	16.2022	10.24	11.11
2.00	2	7.4450	10.52882	7.44500	-87.1527	102.0427	.00	14.89
3.00	2	9.5750	2.66579	1.88500	-14.3762	33.5262	7.69	11.46
Total	6	9.2317	5.08188	2.07467	3.8986	14.5648	.00	14.89

ANOVA

Carrier

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.787	2	5.393	.137	.877
Within Groups	118.341	3	39.447		
Total	129.127	5			