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Research Article

Nasal Carriage of Methicillin-resistant *Staphylococcus aureus* among Elderly People in Lagos, Nigeria

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Abstract

Objectives: *Staphylococcus aureus* is a lethal opportunistic pathogen capable of causing a wide range of infections, especially in debilitated hosts such as the elderly. Nasal carriers of this organism have an increased risk of becoming infected with the pathogen. The purpose of this study was to assess the prevalence of *S. aureus* nasal carriage, to determine the probable risk factors, and to examine the frequency of methicillin-resistant *S. aureus* (MRSA) among elderly people in hospital and nursing home settings in Lagos, Nigeria.

Methods: Two hundred thirty nasal samples were collected from the anterior nares of individuals aged 65 years and older. Possible risk factors were assessed using well-structured questionnaires, and the samples were subjected to standard bacteriological procedures. Antibiotic susceptibility of the isolates was determined with the disk diffusion method. Detection of methicillin resistance was done with the disk diffusion test using cefoxitin 30 μ g, and confirmed with OXOID MRSA CHROMagar.

Results: Fifty (21.7%) *S. aureus* strains were identified among the samples, and antibiotic susceptibility testing showed that multidrug resistance was common. Approximately 20% were resistant to gentamicin, ofloxacin, and mupirocin. Cloxacillin, amoxicillin/clavulanate, and ceftazidime showed the least anti-staphylococcal activity, and almost half of the isolates were resistant to ceftriaxone and cefuroxime. The MRSA nasal carriage rate was 10% and colonization was favored by previous antibiotic use, hypertension, and tuberculosis.

Conclusions: The occurrence of multidrug-resistant *S. aureus* in the elderly cohort indicates their capacity to serve as reservoirs for these strains, which could facilitate the dissemination of MRSA into the community. Therefore, decolonization and the implementation of measures to prevent the spread of this organism are necessary.

Keywords: Antibiotic Resistance, Colonization, Elderly, Risk Factors, Staphylococcus aureus

1. Background

Staphylococcus aureus is one of the commonest human pathogens, capable of causing a wide range of infections in susceptible hosts in communities and hospitals (1). The anterior nares are the principal niche of this organism, from whence endogenous infections can occur. A previous study demonstrated that *S. aureus* blood isolates from septicemic patients were clonally indistinguishable from those obtained from nasal specimens in approximately 82% of patients (2), thereby confirming the association between *S. aureus* nasal carriage and infection. Asymptomatic *S. aureus* carriers may transfer the organism to other members of the community or to susceptible persons, especially those with poor functional status, such as the elderly. The elderly represent a heterogeneous group

with high requirements for healthcare and frequent contact with the general community. Thus, they are at risk of being colonized with different microorganisms, including *S. aureus*, and may be source of transmission of this bacterium from the hospital environment to the community. Among the elderly, *S. aureus* has been implicated as the cause of bacteremia, endocarditis, pneumonia, septic arthritis, and vertebral osteomyelitis (3, 4).

Infections associated with *S. aureus* are often complicated by the remarkable ability of this pathogen to become resistant to various classes of antibiotics and to become more virulent (5). MRSA has been a persistent pathogen in hospitals but its changing epidemiological trend became apparent in the 1990s (6). These strains are resistant to a wide range of antimicrobials due to a penicillin-binding

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protein (PBP2a) encoded for by the *mec*A gene (7). Methicillin resistance in *S. aureus* is detectable phenotypically and genotypically, and cefoxitin disk diffusion and chromogenic agar provide reliable evidence for the identification of *S. aureus* strains (8, 9).

Presently, the proportion of nasal S. aureus isolates identified as methicillin-resistant has increased with variable prevalence data, ranging from Indian figures of 29% in preschool children (10) to 2.8% - 21.6% in long-term care facilities in Hong Kong (11), with an overall prevalence of 21% among healthy individuals in urban and rural communities in Ghana (12). Undoubtedly, MRSA is not simply replacing methicillin-susceptible S. aureus (MSSA) as a causative agent of infections, but is causing an escalation in the incidence of S. aureus infections, thereby adding to the disease burden. As in most other countries, staphylococcal infections have been a major clinical and epidemiological problem in hospitals in Nigeria. MRSA was first noticed in the late 1980s (13). Subsequent data from a number of Nigerian hospitals revealed that approximately 20% -52% of individuals were either colonized or infected with this pathogen (14, 15). MRSA-associated colonization has been described mostly in immunocompetent individuals (16, 17), but colonization of hospitalized and/or immunocompromised patients (18, 19) may lead to invasive infections with possible unfavorable outcomes as a result of the battery of virulence factors possessed by the organism (20, 21). These infections can impose a significant economic burden on individuals and society.

Research evidences from other countries have also addressed the problem of S. aureus carriage in elderly patients in institutional settings or in residents of nursing homes and long-term care facilities (11, 22-24). A report on the impact of S. aureus carriage among elderly patients with end-stage renal disease in Saudi Arabia revealed serious complications and a prevalence of 38.05% (78/205) for *S. aureus* nasal carriage; of these strains, 27.3% (56/205) were MSSA and 10.7% (22/205) were MRSA (22). MRSA carriage in the elderly was significantly correlated with the presence of skin lesions, prior hospitalization within the previous six months, and antibiotic exposure within the previous six months. Although some studies in Nigeria have evaluated the staphylococcal carriage rate among certain vulnerable groups (19) and young adults (16), a substantial proportion of data generated on S. aureus epidemiology are restricted to invasive infections (25, 26) and the problem of *S. aureus* carriage is less defined in the elderly. Since the burden of *S. aureus* carriage could be driven by old age (27, 28), and given the high rate of self-medication and the usage of antimicrobials in most African countries (17), we hypothesized that our older population might have limited risk factors for staphylococcal infections but

would likely be important reservoirs of *S. aureus* with respect to antimicrobial resistance.

2. Objectives

Our primary objective was to determine the nasal *S. aureus* carriage rates among elderly individuals in two hospitals and a nursing home in Nigeria. The probable risk factors for nasal carriage were described and the prevalence of MSSA and MRSA and their antibiotic susceptibility patterns were estimated.

3. Methods

3.1. Study Population and Approval

The study was performed among outpatient adult populations in two general hospitals and residents of a nursing home in Lagos, Nigeria, from May to September 2013. The study population comprised males and females aged 65 years and above. The project proposal was approved by the ethics and research grants committee, College of Medicine of the University of Lagos (reference number: CM/COM/08/VOL.XXIV). The hospitals and the nursing home management provided permission for the use of their centers. Verbal informed consent was obtained from all participants after the study was explained to them.

3.2. Data Collection

Several potential risk factors were investigated, using a well-structured questionnaire. These included the common demographic variables of age, gender, and marital status. We evaluated the relationship between *S. aureus* carriage and self-reported health status or a clinical diagnosis of conditions such as hypertension, diabetes mellitus, arthritis, and pulmonary infections. The use of antibiotics in the preceding three months was also examined.

3.3. Sample Collection

Nasal specimens were collected from the participants using sterile cotton wool swabs. The swab-stick was inserted into both nostrils to carefully sample the mucosa of the nasal septum adjacent to the nasal ostium, which is the preferred habitat of *S. aureus* (29). The sterile swab was rotated in the anterior nasal vestibule of each subject and placed into the swab-stick container. All swabs were transported to a laboratory at the College of Medicine, University of Lagos, and processed within six hours of collection.

3.4. Isolation and Biochemical Identification of Isolates

The samples were inoculated aseptically onto mannitol salt agar plates and incubated at 37°C for 24 hours. Suspected *S. aureus* colonies were inoculated onto Mueller-Hinton agar plates to obtain pure cultures. Identification of the isolates was done on the basis of the morphology of the colonies, positive Gram stain reactions, and standard biochemical tests, including catalase, tube coagulase, DNAse, oxidase, and novobiocin tests (30).

3.5. Antibiotic Susceptibility Testing

Susceptibility to mupirocin (20 μ g), ceftazidime (30 μ g), cefuroxime (30 μ g), gentamicin (10 μ g), ceftriaxone (30 μ g), erythromycin (30 μ g), cloxacillin (5 μ g), ofloxacin (5 μ g), and amoxicillin/clavulanate (30 μ g) was determined by the disk diffusion method according to the criteria of the clinical and laboratory standards institute (CLSI) (31). The British Society for Antimicrobial Chemotherapy (BSAC) interpretive criterion was used for *S. aureus* susceptibility to mupirocin (32). All of the antibiotic discs were purchased from Oxoid (United Kingdom). *S. aureus* ATCC 25923 was employed as the control strain.

3.6. Detection of Methicillin Resistance

All S. aureus isolates were screened for methicillin resistance by the disc diffusion method using a 30 μg cefoxitin disc (Oxoid, UK). The isolates were classified as resistant or sensitive based on the standard interpretative chart of the CLSI (31). Methicillin resistance was confirmed by using MRSA CHROMagar plates (OXOID-MRSA; United Kingdom). A strain of MRSA TSB 023 (obtained from the Nigerian Institute of Medical Research, Yaba) was used as a reference strain.

3.7. Statistical Analysis

The collected data were analyzed using Epi Info 3.5 software. Evaluations were carried out using a 95% confidence interval (CI), and P < 0.05 was considered statistically significant. Data were presented as frequencies. The chisquare (χ^2) test was used for comparisons of positive *S. aureus* nasal carriage, related risk factors, and antibiotic susceptibility testing.

4. Results

Two hundred thirty participants aged 65-94 years were screened for nasal carriage of *S. aureus*. Ten of the participants were residents of a nursing home. The statistical analysis of the 230 participants revealed a mean age of 71.5 years (Table 1). Peak participation was observed in the 65-74-year age group. More females (174; 75.7%) participated in

the study and were more likely to be carriers than males [95% CI 18.9 - 30.4] (Table 1). The mean age of the males was 70.7 years and of females was 68.6 years. Twenty-three (10.0%) of the study participants had arthritis, 41 (17.8%) had human immunodeficiency virus (HIV) infection, 15 (6.5%) had tuberculosis, 112 (48.7%) had hypertension, 55 (23.9%) had diabetes, and three (1.3%) had heart disease. Fifty-nine (25.6%) participants were on antibiotics when their nasal samples were collected, while 66 (28.7%) participants had used antibiotics in the previous three months (Table 1).

The overall prevalence of *S. aureus* nasal carriage was 21.7% (50/230). Among the potential risk factors examined for the acquisition of nasal carriage, there was a significant statistical association (P < 0.05) between the isolation rates of *S. aureus* and hypertension, diabetes mellitus, HIV infection, and tuberculosis. Fifty percent of the *S. aureus* isolates were from individuals with HIV infection, some of whom were co-infected with tuberculosis. *S. aureus* nasal carriage rates among the diabetic and HIV-positive participants were higher than in non-diabetics and HIV-negative subjects (Table 1).

From the statistical analysis of those with positive S. aureus cultures, 15 participants reported using various antibiotics: nine had previously used cotrimoxazole, one had used ampicillin, three had used tetracycline, one used metronidazole and one had used ciprofloxacin. Over 70% of the isolates were resistant to erythromycin, ceftriaxone, and cefuroxime. All of the S. aureus isolates were resistant to ceftazidime, amoxicillin/clavulanate, and cloxacillin (Table 2). Resistance to cefoxitin was detected in 10 (20%) of the S. aureus isolates and confirmed as MRSA by their colony appearance on chromogenic MRSA agar. The most prevalent risk factors in individuals with MRSA were diabetes and hypertension, and six had previously used tetracycline or co-trimoxazole. The MRSA strains had high resistance to amoxicillin/clavulanate (100%), cloxacillin (100%), ceftazidime (100%), cefuroxime (100%), erythromycin (90.0%), and ceftriaxone (80.0%). The predominant antibiotype among the MSSA isolates was resistance to cloxacillin, amoxicillin/clavulanate, and ceftazidime, which was observed in 40 (80%) of the isolates (Table 2). Three MRSA isolates were resistant to gentamicin. The only S. aureus strain isolated from nursing home residents was a MRSA, and this was the only MRSA strain resistant to mupirocin (Table 3).

5. Discussion

Nasal carriage of *S. aureus* is a pivotal source of endogenous infections in colonized individuals and of transmission to other susceptible individuals. However, the prevalence of nasal carriage varies among geographical regions, hospital settings, populations, and people with dif-

Table 2. Antibiotic Resistance of the 50 *Staphylococcus aureus* Isolates (MSSA and MRSA)

Antimicrobial Agent	Number of Resistant Strains (%)		Total (n = 50)
-	MSSA (n = 40)	MRSA (n = 10)	
Mupirocin	8 (20.0)	3 (30.0)	11 (22.0)
Gentamicin	8 (20.0)	2 (20.0)	10 (20.0)
Ofloxacin	6 (15.0)	5 (50.0)	11 (22.0)
Amoxicillin/clavulanate	40 (100.0)	10 (100.0)	50 (100.0)
Ceftazidime	40 (100.0)	10 (100.0)	50 (100.0)
Cloxacillin	40 (100.0)	10 (100.0)	50 (100.0)
Erythromycin	27 (67.5)	9 (90.0)	36 (72.0)
Ceftriaxone	34 (85.0)	8 (80.0)	42 (84.0)
Cefuroxime	36 (90.0)	10 (100.0)	46 (92.0)

Abbreviations: MRSA, methicillin-resistant S. aureus; MSSA, methicillin-susceptible S. aureus.

ferent conditions. To our knowledge, this is the first study from our region reporting the S. aureus nasal carriage rate among the elderly. We found an overall prevalence of 21.7% among the study cohort, in contrast to a 31.8% (n = 61) prevalence of clinical S. aureus isolates from hospitalized patients in surgical wards in the same region in southwest Nigeria (20). Other results indicated that HIV-infected patients displayed a 33% rate (124/375) of S. aureus colonization (19). Also, the proportion of S. aureus colonizers observed in this study is lower than that reported in South Eastern Michigan, United States, where a 42.7% S. aureus nasal carriage rate was observed among 213 nursing home residents (33). Suggestions from various studies have indicated that sample quality, culture methods, immunological defects, genetic influences, antibiotic treatment, and minor deformities of the nasal cavity could be responsible for variations in prevalence rates (34). Nevertheless, the observed nasal carriage prevalence is comparable to that of 23.1% (62/268) occurrence seen among Queensland adults (> 59 years) who were above 59 years old (35).

All of our *S. aureus* isolates were multidrug-resistant, with over 70% resistance to ceftriaxone, cefuroxime, ceftazidime, cloxacillin, amoxicillin/clavulanate, and erythromycin. This could be a result of excessive use of antibiotics in our country, which has no stringent antibiotic use policy. The antibiotics tested in this study were among the most commonly used, inexpensive and easily accessible. Researchers investigating *S. aureus* in Nigeria have found that the less-frequently used and more expensive antibiotics, such as vancomycin and rifampin, are not as readily resisted (15). The high level resistance of the isolates to the panel of antibiotics screened in this study panel agrees with the findings of Udobi et al. (36) and Rao et al. (5).

The prevalence of MRSA was 20% among individuals with culture-positive *S. aureus*. MRSA infections may be

very severe in nursing homes, where residents have a higher risk of death from this organism due to its antibiotic resistance (4, 37). In elderly adults, this is an outcome of weaker immune systems (3, 4, 24), leading to burdens of co-morbid diseases and an increased need for healthcare services. Although the individuals sampled in the present study included residents of a nursing home as well as hospital outpatients, the MRSA colonization rate seemed much lower than the 36.1% reported for eight Korean geriatric hospitals (23) and higher than the 17% reported in residents of long-term care facilities in Spain (38). Likewise, Mainous et al. (37) demonstrated that among individuals with S. aureus isolates, those aged 65 years or older had the highest MRSA prevalence (8.28%). Our findings, however, are in line with reports from other international studies (22, 39).

We also observed that carriage of MRSA was independent of the presence of wounds, disabilities, and previous hospitalizations, which contrasts with findings elsewhere (40). Hypertension and tuberculosis were, however, correlated with MRSA colonization. These conditions represent increased complications and may contribute to poor functional status as previously suggested (23). In addition, a significant relationship exists between nasal carriage and previous antibiotic use (P < 0.05). In contrast, among a group of medical students, Adesida et al. (16) found no significant differences in carriage rate based on previous or current antibiotic use. However, other studies have linked MRSA carriage to antibiotic use (37) and concluded that although differences in prescribing patterns between geographic regions may be unknown, older adults have had increased lifetime exposure to antibiotics and are therefore more prone to MRSA colonization and infections.

One of the strategies for controlling the spread of MRSA includes the eradication of MRSA from colonized individuals (decolonization). Mupirocin (pseudomonic acid A) is a topical antibiotic widely used for treating MRSA skin and surgical-site infections and for eliminating nasal colonization of MRSA (40). In this study, 22% of our isolates were resistant to mupirocin. This is in contrast to previous results that demonstrated 100% mupirocin susceptibility among a set of infectious and colonizing strains of S. aureus (21). This is rather unusual, since mupirocin does not appear to be commonly prescribed or administered for the decolonization of S. aureus in Nigeria. An earlier report (41) also showed 0.5% resistance to mupirocin. Our findings suggest that an increased emergence of mupirocinresistant S. aureus is evident in Nigeria and should be a concern. It is also intriguing to note that the only MRSA strain from the nursing home residents in this study was also resistant to mupirocin. Our concern is that these resistant strains could be transmitted within this social circle due

Table 3. Characteristics of the MRSA Strains

Isolate No.	Study Centre	Age	Gender	Previous Use of Antibiotics	Any Disabilities	Presence of Wound	Previous Hospitalization	Underlying Conditions	Resistance Profile
5	I	70	М	Yes (tetracycline)	No	No	No	Hypertension	MUP, CTR, ERY, CXC, OFL, AMC, CAZ, CRX.
7	I	73	F	Yes (tetracycline)	No	No	No	Hypertension	CTR, ERY, CXC, OFL, AMC, CAZ, CRX.
8	I	70	F	No	No	No	No	Nil	MUP, CTR, ERY, CXC, AMC, CAZ, CRX
14	III	65	F	No	No	No	No	Hypertension	MUP, ERY, CXC, AMC, CAZ, CRX
17	I	75	F	No	No	No	No	Hypertension	GEN, CTR, ERY, CXC, OFL, AMC, CAZ, CRX
19	I	84	F	No	No	No	No	Nil	GEN, CTR, CXC, OFL, AMC, CAZ, CRX.
25	I	73	F	Yes (tetracycline)	No	No	No	Nil	ERY, CXC, AMC, CAZ, CRX.
29	II	68	F	Yes (co-trimoxazole)	No	No	No	Tuberculosis	CTR, ERY, CXC, AMC, CAZ, CRX.
32	II	65	М	Yes (co-trimoxazole)	No	No	No	Tuberculosis	CTR, ERY, CXC, OFL, AMC, CAZ, CRX.
39	П	65	М	Yes (co-trimoxazole)	No	No	No	HIV	CTR, ERY, CXC, AMC, CAZ, CRX.

Abbreviations: AMC, amoxicillin/clavulanate; CAZ, ceftazidime; CRX, cefuroxime; CTR, ceftriaxone; CXC, cloxacillin; ERY, erythromycin; GEN, gentamicin; MUP, mupirocin; OFL, ofloxacin; I and II, hospitals; III, nursing home.

to these individuals' high vulnerability.

However, a thorough surveillance program involving procedures for identifying unknown reservoirs of MRSA, monitoring antibiotic-susceptibility patterns of such pathogens, and establishing prudent antibiotic-use guidelines may be necessary. A major limitation of this study is that nursing homes in Nigeria are socially unacceptable due to cultural practices that encourage close family units or communal living. Institutions intended for the promotion of healthy lifestyles for elderly people are almost non-existent. In Lagos, only one nursing home with very few residents was accessible, so we could not obtain a reasonable sample size from this setting. It is also pertinent to mention that the choice of antibiotics tested was based on the fact that these readily available, cheap antibiotics are more appealing to many Nigerians than expensive ones. Hence, information on the susceptibility profiles of our isolates with regard to easily obtainable antibiotics is important for designing a better antibiotic-use policy.

The data presented in this study revealed a significant nasal carriage rate of antibiotic resistant *S. aureus* among our study cohort. In addition, mupirocin-resistant *S. aureus* strains seem to be increasing among our staphylococcal isolates. Since the study cohort represents an interface between hospitals and communities, these individuals may serve as transmission routes for antibiotic-resistant strains into the community, particularly to other vulnerable subjects, including family members and caregivers. Hence, it is important to address the control of antibiotic-resistant *S. aureus* in age-specific populations and to develop new preventive strategies for MRSA colonization in elderly people (both outpatients and nursing home residents). More importantly, it is imperative to in-

stitute a strict policy on antibiotic prescriptions in our country.

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Footnote

Authors' Contribution: Solayide Abosede Adesida designed the study, supervised the experimental work, participated in data analysis, and wrote the manuscript. Abiola Olufunmilayo Okeyide enrolled the participants, performed the experimental work, and wrote the report. Adefunke Abioye assisted with participants' enrolment and the initial laboratory analysis. Ibilola Omolopo participated in primary data collection and laboratory analysis of the isolates. Tenny Obiageli Egwuatu provided laboratory support. Kehinde Olugbenga Amisu edited the manuscript for content. Akitoye Olusegun Coker contributed to designing the study and revised the manuscript. All authors read and approved the final manuscript.

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Table 1. Sociodemographic Parameters of the 230 Participants

Variable	No. (%)	95% CI	P Value
Age group, y			
65 - 74	192 (83.8)	78.4 - 88.4	
75 - 84	28 (12.2)	8.3 - 17.2	
85 - 94	10 (3.9)	1.8 - 7.3	
Gender			
Female	174 (75.7)	69.6 - 81.1	
Male	56 (24.3)	18.9 - 30.4	
Previous stay in nursing home			
No	220 (95.7)	92.1 - 97.9	
Yes	10 (4.3)	2.1 - 7.9	
Visit to medical practitioner in previous 6 months			
No	21 (9.1)	5.7 - 13.6	
Yes	209 (90.9)	86.4 - 94.3	
Use of antibiotics in previous 3 months			0.4
No	164 (71.7)	65.4 - 77.5	
Yes	66 (28.3)	22.5 - 34.6	
Present use of antibiotics			0.33
No	171 (74.3)	68.2 - 79.9	
Yes	59 (25.7)	20.1 - 31.8	
Specific antibiotics			
Amoxicillin	4 (6.1)	1.7 - 14.8	
Ampicillin	4 (6.1)	1.7 - 14.8	
Ampiclox	9 (13.6)	6.4 - 24.3	
Ciprofloxacin	2 (3.0)	0.4 - 10.5	
Metronidazole	7 (10.6)	4.4 - 20.6	
Co-trimoxazole	27(40.9)	29.0 - 53.7	
Tetracycline	13 (19.7)	10.9 - 31.3	
Hospitalized within the previous 3 months			0.41
No	219 (95.2)	91.6 - 97.6	
Yes	11 (4.8)	2.4 - 8.4	
Disability	(,		0.39
No	224 (97.4)	94.4 - 99.0	
Yes	6 (2.6)	1.0 - 5.6	
Presence of wound	- (=-=)	-11- 5.7	0.9
No	228 (99.1)	96.9 - 99.9	
Yes	2(0.9)	0.1-3.1	
Underlying conditions Heart disease	,		0.19
No	227 (98.7)	96.2 - 99.7	
Yes	3 (1.3)	0.3 - 3.8	
Diabetes	- (- /		0.19
No	175 (76.1)	70 - 81.4	
Yes	55 (23.9)	18.6 - 30	
Hypertension	(/		0.79
No	118 (51.3)	44.6 - 57.9	
Yes	112 (48.7)	42.1 - 55.4	
Tuberculosis	112 (40.7)	JJ13	0.21
No	215 (93.5)	89.5 - 96.3	0.21
Yes	15 (6.5)	3.7 - 10.5	
HIV	3 (33)		0.28

No	189 (82.2)	76.6 - 86.9	
Yes	41 (17.8)	13.1 - 23.4	
Arthritis			0.42
No	207 (90.0)	85.4 - 93.6	
Yes	23 (10.0)	6.4 - 14.6	
Presence of S. aureus			0.21
No	180 (78.3)	72.4 - 83.4	
Yes	50 (21.7)	16.6 - 27.6	
Presence of S. aureus			
Female	40 (80.0)	74.4 - 75.7	
Male	10 (20.0)	24.3 - 25.6	
Presence of MRSA			0.00
No	220 (95.7)	92.1 - 97.9	
Yes	10 (4.3)		