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The Incidence of Beta Lactamase Positive *Staphylococcus Aureus* Among Medical and Non - Medical Students in the University of Jos

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SUMMARY

The incidence of nasal carriage of beta-lactamase positive *Staphylococcus aureus* among medical and non-medical students was determined.

A total of two hundred swab samples were collected from 100 medical and 100 non-medical students at the University of Jos. The swabs were used for the isolation of *S. aureus*. Beta-lactamase production and in vitro sensitivity testing using penicillin were carried out on the isolates.

A total of 87 isolates made up of 45 (93.89%) and 42 (84%) from medical and non-medical students respectively were found to be resistant to penicillin. Eighty-four (96.6%) of the resistant isolates produced beta-lactamase. Approximately 83% and 86% of nasal carriers of *S. aureus* from the medical and non-medical groups respectively, had used one or more antibiotics within a period of three months while 16.7% of medical students and 14% of non-medical students from which *S. aureus* was isolated had used no antibiotics within the same period.

In conclusion, both medical and non-medical students are nasal carriers of *S. aureus* that are primarily penicillin resistant because of the production of beta-lactamase. However, a small number 3 (3.4%) of resistant isolates was found to be beta-lactamase negative, which suggests that other mechanisms of resistance also exits.

INTRODUCTION

The widespread use of penicillin in the treatment of *S. aureus* infections have resulted in a proliferation of strains resistant to this drug world wide. Approximately 70-90% of *S. aureus* isolates from communities in the United States (1) and more than 80% of strains from clinical isolates

in Britain (2) produce penicillinase (beta-lactamase) and are resistant to penicillin.

In Nigeria, the incidence of penicillin resistant *S. aureus* ranges from 83% to 97.8% (3,4). The resistance of *S. aureus* to penicillin is due primarily to the production of beta-lactamase, the exception being methicillin resistant strains which are intrinsically insensitive (5). Healthy nasal carriers play an important role in the epidemiology of *S. aureus* infections.

Prevalence of nasal carriage is influenced by age, race, antibiotics usage and population studied (6).

Since nasal carriage is influenced by several parameters, including population involved, this study was carried out in order to compare the incidence of nasal carriage between medical and non-medical students at the University of Jos.

MATERIALS AND METHODS

Sources and collection of samples

Two hundred healthy students between the ages of 21 and 30 years from the University of Jos were subjects of this study.

The students were divided into two groups: Group one consisted of 100 medical students; 86 males and 14 females. Group two was made up of 100 non-medical students; 40 males and 60 females. Nasal samples were collected by swabbing both nostrils of each student with sterile cotton swabs (Evepon sterile hospital swabs; Evepon Industries Limited, Nigeria).

Isolation and identification of S. aureus isolates

The swabs were streaked on mannitol salt agar plates (on the same day as sample collection) for the isolation of *S. aureus*. The inoculated plates were incubated at 37°C for 48 hours. After incubation, the plates were examined for bacterial growth. Colonies showing typical growth of *S. aureus* (yellow colonies) are picked and transferred onto nutrient agar slants and incubated overnight at 37°C. The isolates were then identified according to methods described by Duguid (2).

Antibiotic Susceptibility Tes'ing

Preparation of discs

Benzyl penicillin solution (Vital Pharmaceutical Ltd. London) was diluted with sterile distilled water to give a final concentration of 100 iu/ml. One ml of the diluted solution was dispensed into a bijou bottle containing 100 (approximately 6 mn) sterile filter paper (Whatman No .1) discs. Each disc was incorporated with benzyl penicillin at a concentration of 10 iu. The discs were dried at 37°C and stored at 5°C until ready for use.

Sensitivity testing

Penicillin sensitivity was carried out using the Bauer-Kirby discs diffusion method (7). *S. aureus* isolates to be tested and control *S. aureus* (NCTC) 6571) were grown overnight at 37°C in nutrient broth. The overnight cultures were then diluted with sterile saline (0.85% NaCl) to yield approximately 10^5 colony-forming units (CFU) per ml. Suspension used as inocula were compared with a standardised barium sulfate suspension according to the method of Vandepitte *et al* (8). Muller-Hinto agar plates were inoculated with test isolates and control strain according to the method of Scott (9). The prepared discs were applied onto the inoculated plates and incubated overnight at 37°C. The results are recorded in Table 1.

Test for beta-lactamase production

Heavy suspensions (about 10^9 CFU/ml) of overnight nutrient broth cultures of staphylococcal isolates and controls (beta-lactamase positive and beta-lactamase negative *S. aureus* strains) were used. Suspensions used as inocula were compared with a standardised barium sulfate suspension (8). Beta-lactamase production was determined according to the audiometric method of Sykes (10).

Statistical analysis

The Chi-square test was used to compare the relatedness or independence of the data obtained.

RESULTS

The incidence of *S. aureus* nasal carriers among students and susceptibility of isolates to benzyl penicillin is recorded in Table 1. Nine (64.3%) female and 39 (45.3%) of male medical students were found to be nasal carriers.

Eight (88.9%) and 37 (94.9%) of *S. aureus* isolates from female and male medical students respectively were found to be resistant to penicillin. Values for the non-medical group ranged from 14 (82.4%) of isolates from males to 28 (84.8%) females.

The results of beta-lactamase production are recorded in Table 2. 44 (97.88%) of resistant isolates from medical and non-medical students respectively produced beta-lactamase. Only 1 (2.2%) of resistant isolate from a medical student and 2 (4.8%) from non-medical students were beta-lactamase negative.

Data relating to frequency of isolation of *S. aureus* to usage of antibiotics are recorded in Table 3. 40 (83.3%) and 43 (86%) of medical and non-medical students respectively reported using one or more antibiotics within a period of three months. 8 (16.7%) and 7 (14%) of medical and non-medical students respectively had used no antibiotics within the same period. This result however, is not statistically significant ($p > 0.05$).

Table 1: Incidence of *S. aureus* nasal carriers among students and resistance of isolates to penicillin

Source	Number of samples collected	Number (%) positive for *S. aureus*	Number (%) of isolates resistant to penicillin
Medical Students			
Female	14	9(64.3)	8(88.9)
Male	86	39(45.3)	37(94.9)
Total	100	48(48)	45(93.8)
non-medical students			
Female	60	33(55.0)	28(84.8)
Male	40	17(42.5)	14(82.4)
Total	100	50(50.0	42(84.0)
Grand Total	200	98.(49.0	87(88.8)

Table 2: Production of beta-lactamase by *S. aureus* isolates

Sources	Number (%) resistant isolates, beta-lactamase positive	Number (%) resistant isolates beta-lactamase negative
Medical Students	44(97.8)	1(2.2)
Non-medical students	40(95.2)	2(4.8)
Total	84(96.6)	3(3.4)

Table 3: Frequency of isolation of *S. aureus* compared with antibiotic usage

Source	Number (%) students *S. aureus* was isolated from	Number (%) using one or more antibiotics	Number (%) using no antibiotics
Medical Students	48(48.0)	40(83.3)	8(16.7)
non-medical students	50(50.0)	43(68.0)	7(14.0)
Total	98(49.0)	83(84.0)	15(15.3)

(P = 0.05)

$X^2 = 0.1236$

X^2 0.05 = 3.841

df 1.

DISCUSSION

Colonization of the anterior nares of humans by *Staphylococcus aureus* occurs in 40% to 50% of individuals (1). Finding from this study is therefore compatible with this value since 49% of the students tested were found to be nasal carriers. The incidence of carriage of *S. aureus* for the females from each student group was higher than the males from the respective groups. This sex-dependent variation in carriage rates has also been reported (6).

It was observed that 83.3% and 86.0% of nasal carriers from the medical and non-medical groups respectively has used one or more antibiotics within a period of three months. In contrast, only 16.7% and 14.0% of medical and non-medical students respectively who were nasal carriers of *S. aureus* had not used antibiotics. This result supports earlier findings that relationship exist between nasal carriage of *S. aureus* and usage of antibiotics. For example, Casewell and Hill (6) stated that nasal carriage is influenced by exposure to antibiotics, while Crossly *et al* (11) found that the acquisition of methicillin resistant *S. aureus* is related to previous administration of two or more antibiotics.

However, more work is required in order to find out exactly how multiple usage of antibiotics contributes to nasal carriage of *S. aureus*.

Approximately 97% of penicillin resistant *S. aureus* isolates also produce beta-lactamase. This result agrees with reported data from other workers (1-3) where resistance to penicillin is attributed primarily to the production of beta-lactamases. A small number 3 (3.4%) of resistant isolates was found to be beta-lactamase negative. This figure is four times higher than that reported by Les Baillie (12) who found only 0.8% prevalence rate for penicillin resistant beta-lactamase negative *S. aureus*.

Most of the resistance of *S. aureus* to penicillin is mediated by the beta-lactam inactivating enzyme (beta-lactamase). However, other mechanisms of resistance also exist. The results from this study as well as other reported works (12,13) suggest resistance mechanisms other than beta-lactamase, as being responsible for the small number of penicillin resistant beta-lactamase negative strains isolated.

The prevalence of penicillin resistant *S. aureus* isolates was slightly higher among medical students than their non-medical counterparts. A probable explanation is that the medical students frequent the hospital environment more than the non-medical students. Since multiple antibiotic resistant staphylococcal are endemic in most hospitals world-wide, they are widely disseminated among staff and patients (13-15).

It is apparent from this study that both medical and non-medical students are nasal carriers of *S. aureus* strains that are mainly penicillin resistant. The trend where most *S. aureus* isolates from both hospital and the general community are resistant to penicillin appears to occur in other parts of Nigeria (3,4) as well as world-wide (1,2).

A probable reason for this trend is that in most developing countries

like Nigeria, antibiotics are freely available on the open market and are often taken without medical advice (13). Hence the total consumption of antibiotics is frequently enormous, misuse of antibiotics for treatment or prophylaxis is generally regarded as a primary factor in the development of antibiotic resistance in human pathogens.

A serious consequence of widespread antibiotic resistance is that it limits the therapeutic effectiveness of antibiotics against pathogenic microorganisms that are initially resistant to them, or that acquire a transferable resistance from another microorganism in the patients' flora during treatment. Curbing the emergence of resistant pathogens and finding newer drugs to treat already resistant strains presents a challenge for both developed and developing countries.

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REFERENCES

1. Jawetz, E., Melnick, J.L., and Adelberg, A.E. (1984). Pyogenic Cocci, In: *Review of Medical Microbiology* (16th ed.). Lange Medical Publication, Los Altos California. pp. 197 - 215
2. Duguid, J.P. (1989). Staphylococcus: Cluster-forming.
3. Cocard, P. (1959). The incidence of antibiotic resistant Staphylococci isolated at Ibadan, 1959 - 1958 West African J. Med. 8 197 - 202.
4. Paul, M.O. Lami, K.A. Aderibigde, D.A. (1982). Nasal Carriers of Coagulase positive Staphylococci in Nigerian Hospitals, Community Trans, Soc. Trop. Med. Hyg. 76: 310 - 322.
5. Basker, M.J. Edmondson, R.A. and Sutherland, R. (1980). Comparative Stabilities of Penicillin and Cephalosporins to Staphylococcal beta-lactamase and activities against *Staphylococcus aureus* J. Antimicrob. Chemother. 6: 33-341.
6. Casewell, M.W. and Hill, L.R. (1986). The Carrier State: Methicillin - Resistant *Staphylococcus aureus* J. Antimicrob. Chemother. 18: 1-12.
7. Bauer, A.W., Kirby, M.N. Sherris, J.C. and Truck M. (1966). Antibiotic susceptibility testing by a standard single Disc Method. Am. J. Clin. Pathol. 45: 493 - 496.
8. Vandepitte, J., Engback, K., Piot, P. and Heuk, C.C. (1991) Basic Laboratory Procedures in Clinical Bacteriology, WHO pp. 85.

9. Scott, A.C. (1989). Laboratory Control of Antimicrobial therapy. In: *Mackie and McCartney Practical Medical Microbiology* 13th ed. Collee, J.G. Duguid, J.P., Fraser, A.G. and Marmion B.P. (eds) pp. 161-181, Churchill Livingstone, New York.
10. Skyes, H.B. Methods for detecting beta-lactamases (1989). In: *Mackie and McCartney Practical Medical Microbiology* 13th ed. Collee, J.G. Duguid, J.P. Fraser, A.G. and Marmion, B.P. (eds) pp 9 - 181, Churchill Livingstone, New York.
11. Crossley, K., Loesch D., Landsman B., Mead, K., Chern, M. and State R. (1979). An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. J. Infect. Dis 139: 273 - 279.
12. Les Baillie, A. (1978). A Survey of the incidence of Penicillin resistant beta lactamases negative strains of *Staphylococcus aureus*. Med. Lab. Sci. 44: 285 - 286.
13. World Health Organization. (1983). Antimicrobial resistance: Report on a working group Bull of WHO 61 (3): 383 - 394.
14. Cookson, B.D., Phillips I. (1988). Epidemic methicillin resistant *Staphylococcus aureus* J. Antimicrob. Chemother. 21: 57 - 65.
15. Aeilts, G.D. Scapico, F.L. Canswuti, H.N. Malik, G.M., and Montgomeria, J.Z. (1982). Methicillin - resistant Staphyoccus: Colonization and infection in a rehabilitation facility. J. Clin. Microbial. 16 (2): 218 -223.