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Research Article

ALARMING CARRIER STATUS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AMONG ADOLESCENT LEARNERS- PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF THE ORGANISM

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ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a well established bacterial pathogen of human beings and is reported as a cause of a number of common diseases both in developed and developing countries. Against the fact that the disease burden is moving in logarithmic phase across the globe, the carriage of MRSA (carrier status) by healthy persons further aggrandizes the issue. In this background, a study has been carried out so as to bring out the real-time carrier status (prevalence) of *Staphylococcus aureus* and MRSA especially among adolescent healthy learners of higher education in the region. The antibiotypes by standard antibiotic susceptibility tests also have been ascertained. The study concluded that while a total of 43.94% (*n*= 272) *S. aureus* of the study was found to be carried by healthy adolescent persons, a total of 20.22% (55 of 272) them was determined to be oxacillin resistant-MRSA. Conclusively, the prevalence of carriage of *S. aureus* and MRSA is alarming among the target group.

Keywords: Carrier, *S. aureus*, MRSA, Resistance, Prevalence, Antibiotypes.

INTRODUCTION

Though *Staphylococcus aureus* is a normal flora of the human body, it could cause various infections of the skin and soft tissues, bacterimia, endocarditis, meningitis and pneumonia in both healthy individuals and those with underlying illness opportunistically. With in half a decade from the first introduction of penicillin in 1940, strains of *S. aureus* unaffected by penicillin were reported (Shehabel-Din *et al.,* 2003 and Korn *et al.,* 2001). Similarly, shortly after the introduction of methicillin in 1959 to treat these infections, *S. aureus* isolates that had acquired resistance to methicillin (methicillin resistant *S. aureus*, MRSA) were reported in 1961 (Enright *et al.,* 2002). The literature thus for generated globally brings out an alarming fact that *S. aureus* displays the capability of acquiring resistance against all classes of antibiotics by either horizontal receipt of resistance gene/s from other bacterium or mutation of an existing bacterial gene by itself. Among the resistance patterns, the methicillin resistance trait acquires a lot of significance owing to its close status of the drug of choice for treatment of infections by *S. aureus.* Shapiro *et al.* (2009) reported the resistance of *S. aureus* to new semi synthetic beta lactams (methicillin, oxacillin and flucoloxacillin) known as MRSA which is due to the presence of plasmid DNA.

Against the fact that *S. aureus* (Goslings and Buchli, 2001) could be a common nasal carrier, several environmental factors could further influence an effective carriage status. Several studies worldwide have reported the rate of nasal carriage of *S. aureus* strains varying from 16.8% to 90% (Korn *et al.,* 2001; Silvana *et al.,* 2005 and Hardy *et al.,* 2004). Approximately, while 20% of the

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human population is reported to be colonized by *S. aureus*, 60% could be intermittent carriers and 20% might never carry the microbe. However, Bassetti *et al*. (2004) so early reported that an alarming 30% of the healthy population carry *S. aureus* in their anterior nares. Further, while a highest rate of carriage has been found in newborns, the rates of carriage *S. aureus* tend to decrease with age.

The isolates of staphylococci are easily spread between humans by skin to skin contact (including from animals), by contact with excretions which contain staphylococci, such as saliva or aerosols released during sneezing and coughing. Airborne transmission is important for the dispersal of staphylococci to many different reservoirs from where, via the hands, they can reach nose. *S. aureus* nasal carriers can dispense high loads of *S. aureus* into the environment and may be the source of an outbreak of *S. aureus* infections - a 'cloud' individual (Sherertz *et al*., 2001). Legesse *et al*. (2004) pointed out the fact that proper personal hygiene could help the protection of human from the harmful infectious pathogen through basic general cleanliness of hands, body, etc. The outbreak causing diseases are transmitted through the persons who do not practice good personal hygiene conditions. As a concern, Payne *et al*. (1966) stated that ~ 25% - 30% of healthy people carry one or more strains asymptomatically and infections are mainly endogenous being caused by patients colonizing strain. Transmission occurs by direct contact to a colonized carrier. Against these facts, the present study has been conducted to bring out the regional prevalence of *S. aureus* and MRSA and to emphasize the importance of personal hygiene in preventing self infection and the carrier status among young learners of higher education.

MATERIALS AND METHODS

At the Research Department of Microbiology, M.R. Government Arts College, Mannargudi, Tamilnadu state, India, the study was carried out between September 2014 and August 2016. At first, a survey was carried out which included questions that documented details on the basic personal hygiene as well as general health awareness practiced among the adolescent college students. Further, different nasal and finger tip swabs were collected aseptically with the help of a sterile cotton swab moistened with sterile saline to prevent any irritation to the volunteering participant as well as to facilitate the easiness of bacterial collection if any into the swab (Oslen *et al*., 2012). The swab was introduced in the anterior nasal cavity at a very shallow deep (less than 1 cm) and rotated 4-5 times both clockwise and anticlockwise directions before withdrawal.

Each of the Muller Hinton media inoculated sample was labeled with an identification number and the basic epidemiological information such as age, sex, location, current previous treatment taken etc., were also tabulated appropriately (Brown *et al.,* 2005 and Collee *et al.,* 1996) from each of the volunteer. The identification of *Staphylococcus* spp. isolates was based on direct microscopic examination (Gram's staining), culture and biochemical characteristics. The confirmation of *S. aureus* was done by coagulase test and further by specific biochemical tests. All the isolates were confirmed in parallel by including a standard strain of *S. aureus* MTCC 3160 – which was employed as a control strain. Confirmation of *S. aureus* strains was in accordance with the procedure outlined by the Clinical Laboratory Standards Institute (CLSI, 2007).

Evaluation of antibiotic susceptibility of *S. aureus*

The susceptibility of all the confirmed isolates of *S. aureus* to a standard battery of antibiotics (Hi- Media) was evaluated based on Kirby- Bauer disc diffusion method. A total of fourteen [Penicillin (10 µg), Oxacillin (1µg), Cefoxitin (30 µg), Vancomycin (10 µg), Gentamycin (10 µg), Tobramycin (10 µg), Tetracyclin (30 µg), Ciprifloxacin (5 µg), Levofloxacin (5 µg), Ofloxacin (5 µg), Moxifloxacin (5 µg), Norfloxaxin (10 µg), Gatifloxacin (5 µg) and Co- Trimoxazole (25 µg)] different antibiotics belonging to ten groups at varying concentrations were selected for antibiogram analyses. In addition, *S. aureus* isolates were tested with oxacillin discs to determine any MRSA.

In addition, *S. aureus* were screened for catalase test, coagulase test (slide and tube methods), sugar fermentation, pigment production onto nutrient agar, hemolytic activities on sheep, coat, chicken and human blood agar, biofilm formation and gelatinase activity. Similarly, the Minimum Inhibitory Concentrations (MICs) of oxacillin against MRSA were also determined in addition of anti-biotyping along with the evaluation of various virulence factors such as DNase activity, thermonuclease and phosphotase activity.

RESULTS AND DISCUSSION

Of the 2476 swab samples collected from 619 volunteering learners (116 male and 503 female learners) and processed microbiologically, a total of 1238, 619, 565 and 54 were from anterior nares, palm, index finger and dorsum respectively. Among them, 10.34% (n= 13) and 5.36% (n= 36) of male and female volunteers respectively reported a recent hospitalization. Further, a total of 4.31% (5) of male and 5.76% (29) of female learners were noted with either recent or current use of antibiotics. It was found that mostly female learners were reported with frequent illness when compared with male. Though the geographical location of most of the learners was noted to be different, only 10.66% of them were from Mannargudi and its immediate surroundings.

The prevalence status of *S. aureus* carriage status was determined to be approximately 44% (n= 272; 43.94%) and was noted to carry *S. aureus* either in their anterior nares or palm or in other parts from where the swabs were collected. Of the 272 *S. aureus* isolated, a total of 43 and 229 were differentiated to be from male and female learners respectively. *S. aureus* is an important pathogen associated with nosocomial and community acquired infections. Nasal

colonisation by *S. aureus* has been identified as an important risk factor for infections that could threaten a carrier's life (Mason *et al*., 2001). Kluytmans *et al.* (1997) observed that the carriage status of *S. aureus* differs over time in certain individuals even without the use of antibiotics. To aggrandize, a close sustenance in staphylococcal prevalence among a community (42% in 2008 and 40% in 2009) was reported by Dar *et al*. (2006).

In this study, among the 272 *S. aureus* isolates, 14.33 % (39 of 272) was found to be multi drug resistant and alarmingly, 55 isolates (20.22%) were identified to be MRSA - methicillin resistant *S. aureus* based on their growth with oxacillin and cefoxitin discs (the diameter of zone of inhibition ≤10 mm and ≤21 mm with oxacillin and cefoxitin discs respectively). About 12% and 5% of *S. aureus* and MRSA respectively from nasal carrier status in community settings were reported by Sharma *et al.* (2014). Positively, none of the isolates of the study showed resistance to gentamycin where as only one displayed resistance to tobramycin. Overall, the antibiogram outcomes of the study suggested that pathogens remained sensitive to a number of agents. Specifically, with gentamycin and tobramycin more than 99% of the carrier derived *S. aureus* was found to be susceptible (Table 1).

Nevertheless, against penicillin and gatifloxacin the current study recorded a high level of resistance was recorded. Clearly, as much as 40.80 % (*n*= 111 of 272) and 85.29 % (*n*= 232 of 272) were determined to be resistant against gatifloxacin and penicillin respectively. It has been suggested that increased exposure of pathogens to a clinical condition might lead to a possible emergence of multi-drug resistance commonly in due course. With respect to *S. aureus*, it is also likely that the resistance to antibiotics could emerge due to mutation of the organisms as the flexibility of *S. aureus* genome could indeed increase its ability to adapt to selective pressure in the environment (Kuroda *et al*., 2001).

Table 1. The antibiotypes of S. aureus (n= 272) isolated from carrier persons.

No.	*Name of the antibiotic disk*	*Total number and % of antibiotypes*					
		Susceptible		*Intermediate resistance*		*Resistance*	
		No.	***%***	***No.***	***%***	***No.***	***%***
1	Penicillin (P) (10 µg)	40	14.70	0	0	232	85.29
2	Oxacillin(OX) (1µg)	244	89.70	5	1.83	23	8.45
3	Cefoxitin (CXX) (30 µg)	258	94.85	0	0	14	5.14
4	Vancomycin (VA) (10 µg)	222	81.61	35	12.86	15	5.51
5	Gentamycin (GEN) (10 µg)	271	99.63	1	0.36	0	0
6	Tobramycin (TET) (10 µg)	271	99.63	0	0	1	0.36
7	Tetracyclin (TET) (30 µg)	211	77.57	31	11.39	30	11.02
8	Ciprifloxacin (CIP) (5 µg)	241	88.60	13	4.77	18	6.61
9	Levofloxacin (LEV) (5 µg)	261	95.95	4	1.47	7	2.57
10	Ofloxacin (OF) (5 µg)	245	90.07	11	4.04	16	5.88
11	Moxifloxacin (MO) (5 µg)	250	91.91	8	2.94	14	5.14
12	Norfloxaxin (NX)(10µg)	258	94.85	5	1.83	9	3.30
13	Gatifloxacin (GAT) (5 µg)	129	47.42	32	11.76	111	40.80
14	Co-Trimoxazole (COT) (25µg)	250	91.91	8	2.94	12	4.41

Alarmingly, it was brought out that all the isolates of the study (272 *S. aureus* out of 2476 of *Staphylococcus* spp. isolated) were identified to be producing either one or two or all of the virulence factors (haemolysin, coagulase, DNase, thermonuclease and phosphatase productions as well as biofilm formation) that were analyzed in the study.

CONCLUSION

The present study clearly intended to document the status of *S. aureus* carriage among absolutely volunteering learners of higher education by aseptically investigating the cases appropriately during the study period. Any variation in prevalence of carrier *S. aureus* in relation to their sites of colonization as well as the patterns of antibiotic resistance were brought out. As has been stated already (Rajaduraipandi *et al*., 2006; Mulla *et al.*, 2007), the need of a real – time concern about the rapid rise in prevalence of and emerging antibiotic resistance among *S. aureus* is re-underscored through the present study. Further, the current evaluation insisted that a time bound and regional level prevalence documentation is mandatory as the prevalence of MRSA varies in not only the different parts of India but globally. Such an attempt would certainly assist the public health providers in containing the staphyloccoal diseases across the nation.

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REFERENCES

Bassetti, S. Lea Wolfisberg, Barbara Jaussi, R.N., Reno Frei, Marcus F. Kuntze, Manuel Battegay, 2004. Andreas F. Widmer, Carriage of *Staphylococcus aureus* among injection drug users: lower prevalence in an injection heroin maintenance program than in an oral methadone program. *Infect. Control Hosp. Epidemiol.* , 25, 133-137.

Brown, D.F.J., Hawkey, P.M., Edwards, D.I., Morrison, D., Ridgway, G.L., Towner, K.J. and Wren, M.W., 2005. Guidelines for the laboratory diagnosis and susceptibility testing of Methicillin Resistant *Staphylococcus aureus* (MRSA). *J. Antimicrob. Chemother.*, 56, 1000- 1018.

CLSI (Clinical and Laboratory Standards Institute), 2007 Performance Standards for Antimicrobial Susceptibility Testing. *Wayne,* 27(1), M100-S17.

Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A. (eds), 1996. Mackie and McCartney Practical Medical Microbiology, 14th edition. Churchill Livingstone, New York, pp. 131-149.

Dar, J.A., Thoker, M.A., Khan, J.A., Ali, A., Khan, M.A., Rizwan, M., Bhat, K.H., Dar, M.J., Ahmed, N., Ahmad, S., 2006. Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India. *Ann. Clin. Microbiol. Antimicrob*., 14(5), 22.

Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann, H. and Spratt, B.G., 2002. The evolutionary history of methicillin resistant *Staphylococcus aureus* (MRSA). *Proc. Natl. Acad. Sci. USA*, 99, 7687-7692.

Goslings, W.R. and Buchli, K., 2001. Nasal carrier rate of antibiotic resistant. *Emerg. Infect. Dis.*, 7, 242-244.

Hardy, K.J., Hawkey, P.M., Gao, F.and Oppenheim, B.A., 2004. Methicillin resistant *Staphylococcus aureus* in the critically ill. *Br. J. Anaesth,*. 56, 121-30.

Kluytmans, J., Van Belkum, A. and Verbrugh, H., 1997. Nasal carriage of *Staphylococcus aureus* epidemiology, underlying mechanisms and associated risks. *Clin. Microbiol. Rev.*, 10, 505-520.

Korn, G.P., Martino, M.D., Mimica, L.J., Chiavone, P.A., Musolino, L.R., 2000. Hih frequency of colonization and absence of identifiable risk factors for methicillin resistant *Staphylococcus aureus* (MRSA) in intensive care units in Brazil. *Braz. J. Infect. Dis.*, 5, 1-7.

Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., Cui, L., Oguchi, A., Aoki, K., Nagai, Y., Lian, J., Ito, T., Kanamori, M., Matsumaru, H., Maruyama, A., Murakami, H., 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. *Lancet*, 357(9264), 1225-1240.

Mason, W.J., Blevins, J.S., Beenken, K., Wibowo, N., Ojha, N., Smeltzer, M.S., 2001. Multiplex PCR protocol for the diagnosis of *staphylococcal* infection. *J. Clin. Microbiol.*, 39, 3332-3338.

Mulla, S., Patel, M., Shah, L. and Vaghela, G., 2007. Study of antibiotic sensitivity pattern of methicillin-resistant *Staphylococcus aureus*. *Indian J. Crit. Care Med.*, 11(2), 99-101.

Oslen, K., Falch, B.M., Danielsen, K., Wilsgaard, T., Sangvik, M., Sollid, J.U.E., Thune, I., Grimnes, G., Jorde, R., Simonsen, G.S. and Furberg, A.S., 2012. *Staphylococcus aureus* nasal carriage is associated with serum 25-hydroxyvitamin D levels, gender and smoking status. The Tromso Staph and Skin Study. *Eur. J. Clin. Microbiol. Infect. Dis.*, 31(4), 465-475.

Payne, M.C., Wood, H.F., Karakawa, W. and Gluck, L., 1966. A prospective study of *Staphylococcal* colonization and infections in new borns and their families. *Am J. Epidemiol.,* 82, 05-16.

Rajaduraipandi, K., Mani, K.R., Panneerselvam, K., M., Bhaskar, M. and Manikandan, P. 2006. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian J. Med. Microbiol.*, 24, 34-38.

Shapiro, A.R., Raman, S., Johnson, M., and Piehl, M., 2009. Community acquired MRSA infections in North Carolina children: Prevalence, antibiotic sensitivities and risk factors. *N. C. Med. J.*, 70: 102-107.

Shehabel-Din, S.A., El-Hadidy, M., Bahaa El-Din, A., El-Hadidy, M. and Zaghloul, H., 2001. Methicillin resistant *Staphylococcus aureus***:** a problem in the burns unit. *Egypt J. Plast. Reconstr. Surg.*, 27,1-10.

Sherertz, R.J., Bassetti, S. and Bassetti-Wyss, B., 2001. "Cloud" health-care workers. *Emerg. Infect. Dis.*, 7(2), 241-244.

Silvana, M., Emmanuel, R. and Carlos, C., 2005. Prevalence of *Staphylococcus aureus* introduced into intensive are units of a university hospital. *Braz. J. Infect. Dis.*, 9: 1-12.