International Journal of General Medicine and Pharmacy (IJGMP)
ISSN 2319-3999
Vol. 2, Issue 3, July 2013, 1-8

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

PREVALENCE OF COMMUNITY ACQUIRED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*NASAL COLONIZATION IN SCHOOL CHILDREN

MUSTAFA MURTAZA, L. LUKAS LYBIANA & M. ZORAIRAH

School of Medicine University Malaysia, Sabah, Kota Kinabalu, Sabah, Malaysia

ABSTRACT

Methicillin resistant *Staphylococcus aureus* (*MRSA*) has emerged as a community acquired infection in the past decade. The cause of the increasing incidence of CA-MRSA infection in previously healthy hosts is not completely understood. From October 2012 to November 2012, a total of 388 children ages between 7 years to 12 years, were screened for carriage of MRSA .The overall prevalence of nasal carriage of *MRSA* and *Staphylococcus aureus* were 4 % and 32 % respectively. About 196 (51%) males and 192 (49 %) females. About 284 (73.0%) were ethnic Bajau, 67(17.0 %) ethnic Malay and remaining other races. *MRSA* and *S.aureus* colonization was higher in ethnic Bajau 9.0 % and 65.0 % respectively.*MRSA* colonization was higher in the children ages > 7 to 9 years 7 % but *S.aureus* colonization was higher (50 %) in the children ages > 10 to 12 years. All 146 isolates were susceptible to vancomycin, ciprofloxacin, and gentamicin .*MRSA s*howed moderate resistance to ceftazidime.All isolates showed moderate to high resistance to erythromycin, penicillin and ampicillin respectively.

KEYWORDS: *Staphylococcus aureus*, Colonization, MRSA, CA-MRSA

INTRODUCTION

Staphylococcus aureus is a normal flora of nasal, skin, groin, perineum and axilla. Colonization with *S.aureus* has been identified as an important risk factor for the development of *S.aureus* infections in both community and hospitalized setting [1,2].The modern era of antimicrobial therapy begin in 1940s with the introduction of penicillin and first beta lactamase such as cephalosporin's and semisynthetic methicillin became available in the late 1950s.First methicillin resistant *Staphylococcusaureus* (MRSA) was described at about same time[3].The MRSA originally confined to the hospital environment, now MRSA has emerged as a community –acquired infection over the last decade[4-6].The Community acquired MRSA (CA-MRSA) is different from hospital acquired MRSA from both epidemiological and molecular points of views. Case-definition studies showed that hospital or health-care associated MRSA (HCA-MRSA) and CA-MRSA represented different organisms that produced different clinical syndromes [7,8].CA-MRSA is an emerging pathogen with an epidemiology and pathogenesis that continue to be defined [9-11]. A recent meta-analysis of studies addressing CA-MRSA colonization in various communities demonstrated a prevalence of 1.3 %[7].In specific populations, hospital admission within 12 months has been noted to be a risk factor for CA-MRSA carriage; however ,genotypic characteristics of CA-MRSA suggest that most strains have not originated from hospitals [12,13].The virulence of CA-MRSA may not be limited solely to its resistance to beta lactam antimicrobial drugs.CA-MRSA appears to have a distinct exotoxin genetic armamaritarium- in particular Panton-valentine leukocidin (PVL) locus which has been associated with severe infections [14-17].

The aim of this retrospective study was to determine prevalence of CA-MRSA nasal colonization in primary school children.

SUBJECTS AND METHODS

Design

Retrospective study was conducted to determine the CA-MRSA nasal colonization prevalence in primary school children.

Study Area-Setting

The study was conducted in the National Primary school Big Pengalat,Papar District of Sabah ,Malaysia .Big Pengalat Primary school with six hundred children

Ethic Clearance

Prior permission from Malaysian Ministry of Education, Director Education, Sabah and ethic clearance was obtained.

Nasal Specimen Collection

Primary school children age 7 to 12 years was involved in the study. Informed consent of parent or guardian was obtained. Subjects on active antibiotic therapy, past two weeks antibiotic therapy or hospitalization were excluded. A demographic detail of subjects was recorded in the study protocol. After all details completion nasal swab were collected with Becton Dickinson culture swabs with Stuart media (BD220093)

Microbiological Identification and Antimicrobial Susceptibity Tests

All specimens were cultured within three hours of collection. Columbia blood agar was used as primary culture,mannitol salt agar(BD),DNase test agar(BD), rapid Latex agglutination test for presumptive coagulase test ,Avipath Staph,OD044 kit (Omega Diagnostic,UK) and Gram staining was used for identification.Antibiotic susceptibility test of *Staphylococcus aureus*isolates were testedby Bauer-Kirby disc diffusion techniques (1966) on Muller Hinton agar(BD), according to NCCLS guidelines. Antibiotic disc used in thisassayincludedpenicillin(10ug), ampicillin(10ug), erythromycin(10ug), gentamicin(30ug), cefuroxime(30ug)ceftazidime(30ug), refapimin(5ug), ciprofloxacin(5ug), vancomycin(30ug). The antibiotic assay included tests on sensitive control organism with batch of test strain *Staphylococcus aureus* ATCC 25923.. Confirmation of methicillin resistance in *Staphylococcus aureus*-resistance to methicillin was examined by the method described by Barber (1964).

Identification of MRSA was tested by detection of methicillin resistant determinant (mecA) product, penicillin binding protein 2 (PBP2) with rapid slide latex agglutination test for identification of MRSA was tested by commercial available test kit (Denka Seiken,Japan)..Molecular typing by pulsed –field gel electrophoreses (PFGE) and Panton-valentine leukocidin (PVL) gene testing were not performed in this study. Data was analyzed by using of Social Sciences StatisticalPackage (SPSS 16).The difference between values was considered significant when $P<0.05$ was obtained.

RESULTS

During this study 388 subjects were recruited from Big Pengalat Primary school. The number of subjects in the each age group ranged from 178>7 to 9 years to 210 for children of ages>10 to 12 years. Of 196 (51%) males and192 (49%) females. About 284 (73.0%) were ethnic Bajau,, 67 (17.0%) ethnic Malay and remaining other racial ethnic groups(table 1). Of total 388 subjects enrolled in the study 146 (37.68 %) were colonized with*Staphylococcus*, Of 146 isolates 125 (32.0%) were methicillinsusceptible *Staphylococcus .aureus* (MSSA)Of 146 isolates 15 (4.0%) were CA-MRSA and six (1.5%) demonstrated to be methicillin resistant *Staphylococcus (*MRS).TheMSSA,CA- MRSA and MRS

nasal colonization in ethnic Bajau was higher (65.0%,9.0%, and 3.5 % respectively) than in ethnic Malay (MSSA 11.5 %). The MSSA, CA- MRSA and MRS nasal colonization was significantly higher in the children ages >10 to 12 years (50.0%, 3.0 %, and 2.6 % respectively) than in the children children ages > 7 to 9 years (35.5 %, 7.0%, and 1.0 % respectively) .(Table 2).

Antimicrobial susceptibility testing of 146 isolates indicated resistance pattern suggestive of CA-MRSA,MRS and MSSA.The detailed susceptibility distribution of various antimicrobials for the isolates is shown in(Table 3).All 146 isolates were susceptible to vancomycin, ciprofloxacin and gentamicin.CA-MRSA showed lower susceptibility to rifampicin and, cefuroxime, but moderate resistance to cefatizidime .All isolates showed moderate to high resistance to ampicillin, penicillin and erythromycin respectively.

Table 1: Demographic of the Study Population: 388 Subjects Analyzed

Variables	Age Group (Years)		Gender		Ethnicity				
	7 to 9	10 to12	Male	Female	Bajau	Malay	Kadazan	Brunei	Chinese
Number	178	210	196	192	284	67	28	6	3
Percentage %	46	54	51	49	73	17	7	2	1

Table 2: Age, Gender and Ethnicity Distribution of Nasal Carriage of CA- MRSA, MRS and MSSA among School Children n= 146/388 (37.62)

Characteristics	CA-MRSA n=15(4.0)	MRS n=6(1.5)	MSSA n= 125(32.0)
Age Group			
7 to 9	10 (7.0)	2 (1.0)	52 (35.5)
10 to 12	5 (3.5)	4 (3.0)	73 (50.0)
Gender			
Male	5 (3.5)	3 (2.0)	65 (44.5)
Female	10 (7.0)	3 (2.0)	60 (41.0)
Ethnicity			
Bajau	13 (9.0)	5 (3.5)	95 (65.0)
Malay	0		17 (11.5)
Kadazan	2 (1.0)	1 (0.5)	9 (6.0)
Brunei	0		3 (2.0)
Chinese	0	0	1 (0.5)

Figures in Parentheses Indicate Percentage

Table 3: Antimicrobial Susceptibility Pattern of 146 Colonizing CA- MRSA, MRS and MSSA Isolates from School Children

Pathogens	n. Isolates	Antibiotics Resistance %								
		P	AM	E	GN	CXM	CAZ	CIP	RA	VA
CA-MRSA (4%)	15	67	60	74	100	94	60	100	94	100
MRS (1.5%)	6	84	67	84	100	100	34	100	100	100
MSSA (32%)	125	74	75	94	99	100	71	100	100	100

P, penicillin, AM, ampicillin, E, erythromycin, GN, gentamicin, CXM, cefuroxime, CAZ, ceftazidime, CIP, ciprofloxacine, RA, rifampicin, VA, vancomycin

DISCUSSIONS

Our study indicate that the prevalence ofnasal MRSA colonization among otherwise healthy children in the Big Pengalat primary school was 4.0 % during the period from October 2012 to November 2012.Compared with a study in Taiwan among children of ages between 2 months and 5 years was 7.3 %.[18].In the United States, where CA-MRSA is also being increasingly reported the MRSA colonization prevalence for general population appeared to have been

relatively low until the year 2002 [19,20].In a survey involving 9,622 persons conducted between 2001 and 2002, national *S.aureus* and MRSA nasal colonization prevalence estimates were 32.4 % and 0.8 %, respectively .For healthy children, the nasal colonization was ranged from 0.22 % to 2.2 % [21,22].

As reported in several pediatric studies; however, and increasing trend in this regard has been noted in certain areas of United States recently [23].Creech *et al*. reported that nasal MRSA colonization rate among healthy children in Nashville, TN, increased significantly from 0.8 % in 2001 to 9.2 % in 2004. In the United States between 1998 and 2002 researchers reported a MRSA prevalence of 1.3 %[7].In another study in Taiwan MRSA carriage was 7.8 % among health children from 2005- 2008,with rates ranging from 6.2 % to 9.5 % in different geographic areas[24].Which was considered to be higher thanTaiwanese adult populations surveyed duringthe same period (3.8 %; P<0.0001) [25].The CA-MRSA isolates were initially identified in pediatric populations and subsequently reported in adults population [26]..In a prospective observational study in 812 US Army soldiers 3 % were colonized wit CA-MRSA and 9 of whom (38 %) developed soft-tissue infections during the study period [27].Our observations are comparable to other studies.

There are now compelling data suggesting that the pathogenesis of CA-MRSA is unique and distinct form of HA-MRSA .Rather than using the model of HA-MRSA that asserts that there is stepwise progression from MRSA colonization to MRSA infection [28].Although superficial skin and soft tissue infections remained the most common manifestation of CA-MRSA, severe diseases such as necrotizing pneumonitis, necrotizing fasciitis ,osteomyelitis ,pyomyositis ,septic embolism, and venous thrombosis were not uncommon and previously caused death in healthy children[29,30].The cause of the increasing incidence of CA-MRSA infection in previously healthy hosts are not completely understood, and the factors influencing CA-MRSA virulence remain an issue of ongoing debate[31-33].

Colonization with *S.aureus* has been identified as an important risk factor for the development of *S.aureus* infections in both community and hospital settings {2,34].Evidence further suggests that, compared to methicillin-susceptible *S.aureus* (MSSA),colonization with MRSA imposes significantly greater risk for development of subsequent infections[28].There are several limitations in this study. First, there is no information on the prevalence of CA-MRSA in any past study. Therefore difficult to predict any increase or decrease in prevalence of CA-MRSA colonization in healthy children in this region. Second, our study is retrospective and it lack information if the colonized children developed any superficial or soft tissue infections? Thirdly, molecular typing of CA-MRSA isolates have not been performed and therefore no information if the CA-MRSA isolates belongs to resistant clones of characterized sequence type 59 (ST59) or the clones also possess Panton-Valentine Leukocidin (PVL) genes. Study also lacks information on the family size and information on crowding in the community. Crowding is an independent environmental risk factor that might accelerate CA-MRSA transmission in the community [26].Further study is needed to know more on CA-MRSA and to address some of the limitations in the study.

CONCLUSIONS

In this study, we observed 4. % healthy school children were colonized by *MRSA* in the nares from October 2012 to November 2012.Colonization was more common in ethnic Bajau and in children ages >7 to 9 years. Efforts must be made to prevent *MRSA* spread in the community. Further research is needed to determine the host factors that accelerate spread of *MRSA* in the community.

ACKNOWLEDGEMENTS

We wish to thank Vice Chancellor and the Dean School of Medicine, University Malaysia Sabah for the permission to publish this article.

Financial Support

University Malaysia, Sabahgrants (SBK0050-SKK-2012).

Potential Conflict of Interest

All authors: no conflict.

REFERENCES

1. Huang, Y. C., Y. H. Chou, L. H. Su, R. I. Lien, and T.Y. Lin. Methicillin- resistant *Staphylococcus aureus*colonization and its association with infection among infants hospitalized in neonatal intensive care units. *Pediatrics*2006;118:469-474.
2. vonEiff, C., K. Becker, K. Machka, H. Stammer, and G. Peters. Nasal carriage as a source of *Staphylococcus aureus*bacteremia. Study Group. *N. Engl. J.* Med.2001;344:11-16.
3. Jevons MP. "Celbenin" resistant*Staphylococcus.Br Med J.*1961; 1:124-125.
4. Dufour P, Gillete Y, Bes M*et al*. Community acquired methicilin resistant *Staphylococcus aureus* infections in France: emergence of a. clone that produces Pantong Valentine leukocidin. *Clin InfectDis*.2002;35:819-824.
5. Moran GJ, Krishnadasan A, Gorwitz RJ, *etal.* Methicillin-resistant *S.aureus*lnfections among patients in the emergency department .*N.Engl J Med*.2006;355:666-674.
6. Dietrich DW, Auld D, MermelLA .Community-acquired methicillin-resistant *Staphylococcus aureus* in southern New England Children.*Pediatrics*.2004;113:e347e352
7. Salgado CD, Farr B,Calfee DP. Community acquired methicillin resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors, *Clin InfectDis.*2003; 36:131-139.
8. Diep B, Stone GG, Basuino L, *et al*. The arginine catabolic mobile element and *Staphylococcal* chromosomal cassette *mec*linkage: convergence of virulencein the USA300 clone of methicilin resistant *Staphylococcus aureus. J Infect Dis.*2008; *197 :* 1523-1530.
9. Bukharie HA Abdelhadi MS Ibrahim AS Rubaish AM Larbi EB. Emergence of methicillin-resistant *Staphylococcus aureus*as a community pathogen . *Diag Microbial InfectDis* 2001; 40:1-4.
10. Moreno F, Crisp C, Jorgensen JH, Patterson JE. Methicillin –resistant *Staphylococcus aureus*as a community organism. *Clin InfectDis* 1995; 21: 1308-12.
11. GorakEJ , Yamada SM , Brown JD, Community acquired methicillin resistant *Staphylococcus aureus*in hospitalized adults and children without known risk factors. Clin*Infect Dis* 1999;29:797-800.
12. Charlebois E.D,Bangsberg D.R, Moss N.J,*et al.* Population-based prevalence of methicillin resistant *Staphylococcus aureus*in the urban poor of san Francisco. *Clinlnfect Dis* 2002;34:425-33.
13. Jernigan JA, Pullen AL, Partin C, Jarvis WR. Prevalence of and risk factors for colonization with methicillin-resistant *Staphylococcus aureus*in an outpatient clinic population. *Infect Control HospEpidemiol* 2003;24:445-50.
14. Naimi TS, LeDell KH, Como-Sabetti K, *et al*. Comparison of community-and health care- associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003;290:2976-84.

15. Fey PD, Said-Salim B Rupp ME, *et al*.Comparative molecular analysis of community-or-hospital-acquired associated methicillin-resistant *Staphylococcus aureus. AntimicrobAgents Chemother* 2003;47:196-203.

16. Daum RS Ito T Hiramatsu K, *et al*. A novel methicillin-resistant cassette in community-acquired methicillin-resistant *Staphylococcus aureus*isolates ofdiverse genetic background. *J Infect Dis*.2002; 186:1344-7.

17. Mongkolrattanothai K, Boyle S, Kahana MD, Daum RS. Severe *Staphylococcus aureus*infections caused by clonally related community-acquired methicillin susceptible and methicillin –resistant isolates. *Clin Infect Dis*.2003;37:1050-8.

18. Huang Y.C., Hwang K. P., Chen P.Y., Chen C.J., and Lin T.Y. Prevalence of methicillin-resistant *Staphylococcus aureus*Nasal colonization among Taiwanese children in 2005 and 2006. *J. Clin. Microbiol*.Dec 2007.45:12:3992-3995

19. Hussain, F. M., S Boyle- Vavra, C.D. Bethel, and R. S. Daum. Community- acquired methicillin resistant *Staphylococcus aureus*colonization in healthy children attending an outpatient pediatric clinic. *Pediatr. Infect. Dis.J*.2001; 20: 763-767.

20. Kuehnert, M. J., D. Kruszon-Moran, H. A. Hill, *et al*. Prevalence of *Staphylococcus aureus*nasal colonization in the United States, 2001-2002.*J. Infect. Dis.*2006;193: 172-179.

21. Nakamura, M. M., K. L. Rohling, M. Shashaty, H. Lu, Y. W. Tang, and K. M. Edwards. Prevalence of methicillin-resistant *Staphylococcus aureus*nasal carriage in the community pediatric population, *Pediatr. Infect. Dis. J*. 2002;21:917-922.

22. Shopsin, B., B. Mathema, J. Martinez, *et al.* Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*in the community. J. Infect. Dis. 2000;182:359-362.

23. Creech, C. B., II, D. S. Kernodle, A. Alsentzer, C. Wilson, and K. M. Edwards. Increasing rates of nasal carriage of methicillin-resistant *Staphylococcus aureus*in healthy children. *Pediatr. Infect. Dis. J*.2005;24:617-621.

24. Chih J.C., Kuang H. H., Tzou Y. L., Kao P.H., Poh Y. C., and Yhu C. H. Factors associated with nasal colonization of methicillin-resistant *Staphylococcus aureus*among healthy children in Taiwan. *J.Clin. Microbiol*.2011;.49:131-137.

25. Wang, J. T., *et al.*Prevalence of and risk factors for colonization by methicillin-resistant *Staphylococcus aureus*among adults in community settings in Taiwan. *J. Clin. Microbiol.* 2009*:*47:2957-2963.

26. Herold,B.C., *et al*. Community-acquired methicillin-resistant *Staphylococcus aureus*in children with no identified predisposing risk. 1998;*JAMA*279:593-598.

27. Ellis, M. W., D. R. Hospenthal, D.P.Dooley, P.J. Gray, and C.K. Murray.Natural history of community-acquired methicillin-resistant *Staphylococcus aureus*colonization and infection in soldiers. *Clin. Infect. Dis*.2004;39: 971-979.

28. Mulligan ME, Murray-Leisure KA, Ribner BS, *et al*. Methicillin-resistant *Staphylococcus aureus:*a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med*1993;94:313-28.

29. Chen, C.J., *et al*. Clinical features and molecular characteristics of invasive community-acquired methicillin-

resistant *Staphylococcus aureus*infections in Taiwanese children. *Diag. Microbiol. Infect.*2007;59:287-293.

30. Gonzalez, B.E., *et al*.Severe Staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant *Staphylococcus aureus.Pediatrics*2005;115:642-648.

31. Boyle-Vavra, S., and R.S. Daum. Community-acquired methicillin-resistant *Staphylococcus aureus* : the role of Panton- Valentine leukocidin. *Lab. Invest*. 2007;87: 3-9.

32. Deleo, F.R., M. Otto. B. N. Kreiswirth. and H. F. Chambers.community-associated methicillin-resistant *Staphylococcus aureus.Lancet*.2010;375:1557-1568.

33. Otto M. Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus. Annu*. *Rev. Microbiol*.2010;64 :143-162.

34. Huang, Y.C., Y.H. Chou, L. H. Su, R. I. Lien, and T.Y. Lin. Methicillin-resistant *Staphylococcus aureus*colonization and its association with infection among infants hospitalized in neonatal intensive care units. *Pediatrics.* 2006;118: 469-474.