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Article/Artigo

Community prevalence of methicillin and vancomycin resistant *Staphylococcus aureus* in and around Bangalore, southern India

Prevalência de *Staphylococcus aureus* resistente à meticilina e à vancomicina em comunidade no entorno de Bangalore, Índia do Sul

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ABSTRACT

Introduction: *Staphylococcus aureus* is a known colonizer in humans and has been implicated in community acquired soft tissue infections. However emergence of methicillin resistant *S. aureus* (MRSA) has aroused great concern worldwide. This study aimed to determine the prevalence of MRSA in the community of Bangalore, southern India. **Methods:** Swabs were collected from anterior nares, forearm, dorsum and palm of the hands of 1,000 healthy individuals residing in and around Bangalore, belonging to different socioeconomic strata and age groups. **Results:** Analysis verified that 22.5% and 16.6% of the individuals presented *Staphylococcus aureus* and MRSA, respectively, at any of the three sites. Vancomycin resistance was observed in 1.4% of the *S. aureus* isolates, which was confirmed by detection of the *vanA* gene. It was interesting to note that 58.8% of the children in the age group 1-5 years-old presented MRSA, the highest percentage compared to other age groups of < 1 (44.4%) year-old, 5-20 (21.7%) years-old, > 40 (11%) years-old and 20-40 (9.9%) years-old. Among the population of various socioeconomic strata, maximum MRSA colonization was observed among doctors (22.2%), followed by upper economic class (18.8%), lower economic class (17.7%), apparently healthy hospital in-patients (16.5%), nurses (16%) and middle economic class (12.5%). Most of the MRSA isolates were capsular polysaccharide antigen type 8 (57.1%). **Conclusions:** There is a need for continuous surveillance and monitoring of the presence of MRSA in the community and a clearer understanding of the dynamics of the spread of MRSA will assist in controlling its dissemination.

Keywords: *Staphylococcus aureus*. Methicillin resistance. Vancomycin resistance. Healthy population. Colonization.

RESUMO

Introdução: O *Staphylococcus aureus* é conhecido por ser um colonizador em humanos sendo implicado em infecções comunitárias dos tecidos moles. Contudo, a resistência à meticilina e emergência de *S. aureus* meticilina resistentes (MRSA) têm despertado preocupação em todo o mundo. O presente estudo visa encontrar a prevalência de MRSA na comunidade de Bangalore, sul da Índia. **Métodos:** *Suabes* foram coletados de narinas anteriores, antebraço e dorso da palma de 1.000 indivíduos saudáveis, residentes em Bangalore e nas proximidades, pertencentes a diferentes estratos socioeconômicos e faixas etárias. **Resultados:** Observou-se que 22,5% e 16,6% dos indivíduos foram abrigar *Staphylococcus aureus* e MRSA, respectivamente, em qualquer um dos três locais. Dos *S. aureus* isolados, 1,4% também foram resistentes à vancomicina, o que foi confirmado pela detecção do gene vanA. Foi interessante notar que 58,8% das crianças na faixa etária de 1-5 anos foram abrigar MRSA, o mais elevado em comparação com outros grupos etários de < 1 (44,4%) ano, 50-20 (21,7%) anos, > 40 (11%) anos e 20-40 (9,9%) anos. Entre a população de diferentes estratos socioeconômicos, a colonização de MRSA máxima foi observada entre os médicos (22,2%), seguida pela classe econômica superior (18,8%), classe baixa (17,7%), pacientes aparentemente saudáveis (16,5%), enfermeiros (16%) e classe econômica média (12,5%). A maioria dos MRSA isolados eram do tipo polissacarídeo capsular antígeno 8 (57,1%). **Conclusões:** Há uma necessidade de vigilância e monitorização contínua da presença de MRSA na comunidade, bem como uma melhor compreensão da dinâmica de propagação de MRSA pode ajudar no controle da disseminação.

Palavras-chaves: *Staphylococcus aureus*. Resistência à meticilina. Resistência à vancomicina. População saudável. Colonização.

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INTRODUCTION

Staphylococcus aureus is ubiquitous in nature and a known colonizer in humans. Community acquired soft tissue infections due to *S. aureus* is quite common[1]. Recently, community acquired *S. aureus* has raised concerns due to increasing methicillin resistance. Methicillin resistant *Staphylococcus aureus* (MRSA) infections do not respond to beta-lactam antibiotics and vancomycin is the drug of choice[2-4]. MRSA has been implicated in both community acquired and hospital acquired infections and many of the clinical infections arise from the spread from healthy carriers. The anterior nares are the most common site for colonization, due to moist squamous epithelium and ventilation, and are also responsible for dissemination in the community. Even healthy individuals carrying MRSA present a small risk of contracting an invasive infection.

Thus, it is imperative to determine the prevalence of MRSA as colonizers in the community, as well as presence of vancomycin resistance among them.

METHODS

In between april 2003 to december 2007, 1000 individuals (M:F=3:2) of various Socio-economic status based on the monthly per capita income (MPCI) in Rs. namely upper economic class (MPCI > Rs. 20000) (n=324), middle economic class (MPCI Rs. 5,000-20,000) (n=233), lower economic class (MPCI< Rs. 5,000) (n=181), doctors (n = 27), nurses (n = 144), apparently healthy hospital in-patients (n = 91), belonging to the age groups (< 1 year-old [n = 9], 1-5 years-old [n=24], 5-20 years-old [n = 434]; 20-40 years-old [n = 424] and > 40 years-old [n = 109]), who were not on any antibiotic therapy and residing in the City of Bangalore and the adjacent Kolar district were recruited. Following free informed consent, swabs were collected from the anterior nares for resident flora and from the forearm, dorsum and palm of the hands for transient flora from 1,000 individuals. Transport swabs (Himedia, India) were used for sample collection and transportation to the

laboratory. Culture and identification was performed, according to standard procedures[5]. All the *S. aureus* isolates were screened for methicillin and vancomycin resistance using the modified Kirby Bauer disc diffusion method, according to the NCCLS guidelines[6]. All the vancomycin resistant strains were further confirmed by detecting the *van A* gene by polymerase chain reaction using the primers UNRG-Fp- 5'- CATGAATAGAATAAAAGTTGCAATA-3' and UNRG- Rp- 5' CCCCTTTAACGCTAATACGATCAA described earlier by Tiwari et al[7].

For all the strains of *S. aureus* that were isolated, including MRSAs, capsular antigen typing was performed against specific antisera of type-5 and 8 capsular serotype strains, as previously described by Paul et al[8].

Ethical considerations

The Ethical Review Board of Gulbarga University approved the study. Sampling was performed after obtaining oral consent from the subjects.

RESULTS

From the three anatomical sites of the 1,000 individuals screened, 1,023 coagulase negative *Staphylococci* (CoNS) and 282 *S. aureus* were isolated, of which 205 were MRSA. Among the CoNS, 58.8% were isolated from the anterior nares (n = 588), 26.2% from the forearm (n = 262) and 17.3% were from the dorsum and palm of the hands (n = 173). For *S. aureus*, 22.5% were isolated from the anterior nares (n = 225), 2.4% from the forearm (n =24) and 3.3% from the dorsum and palm of the hands (n = 33). The distribution of MRSA was highest in the anterior nares (16.6%; n = 166), followed by the forearm (2%; n = 20) and the dorsum and palm of the hands (1.9%; n = 9). All the patients presenting *S. aureus* as transient flora on their forearms and the dorsum and palm of the hands also presented *S. aureus* in their anterior nares and thus the individual prevalence of *S. aureus* determined was 22.5%. A similar value was determined for MRSA, with an individual MRSA prevalence of 16.6%.

The distribution of *S. aureus* and MRSA according to age is presented in **Table 1**. The greatest presence of MRSA was observed in the age group 1-5 (58.3%) years-old, followed by < 1 (44.4%) year-old, 5-20 (21.7%) years-old, > 40 (11%) years-old and 20-40 (9.9%) years-old. Although a high percentage of MRSA was determined for resident flora in the age groups < 1 year-old and 1-5 years-old, the percentage of MRSA as transient flora was very low.

The prevalence of *S. aureus* and MRSA according to occupation and socioeconomic status is presented in the **Table 2**. Of some interest was the fact that 22.2% of the doctors presented MRSA as resident flora and 3.7% as transient flora, the highest values for any group. Doctors were followed by individuals from the higher economic class (18.8%), the lower economic class (17.7%), apparently healthy in-patients (16.5%) and nurses (16%), while the lowest values occurred in the middle economic class (12.5%).

Vancomycin resistance was observed in the 1.4% (n = 4) of the *S. aureus* isolates. Vancomycin resistance was confirmed by PCR amplification of the *vanA* gene. All the vancomycin resistant strains were resistant to methicillin, except one.

The presence of type 5 and type 8 capsular antigen types among the *S. aureus* and MRSA isolates is shown in **Table 3**. Capsular antigen type 8 was the most common among both *S. aureus* (54.3%) and MRSA isolates (57.1%) compared to type 5, which showed a presence of 21.6% of *S. aureus* and 21% of MRSA. Among non-type 5/non-type 8 isolates, 24.1% were *S. aureus* and 21.9% were MRSA.

TABLE 1 - Distribution of *Staphylococci* colonization in healthy individuals according to age.

Age groups (years)	*Staphylococcal* isolates	Anterior nares		Forearm		Dorsum and palm		Total
		n	%	n	%	n	%	
< 1	CoNS	1	11.1	7	77.8	3	33.3	11
(n = 9)	*S. aureus*	4	44.4	0	0.0	2	22.2	6
	MRSA	4	44.4	0	0.0	1	11.1	5
1-5	CoNS	2	8.3	14	58.3	10	41.7	26
(n = 24)	*S. aureus*	17	70.8	3	12.5	2	8.3	22
	MRSA	14	58.3	2	8.3	0	0.0	16
5-20	CoNS	171	39.4	71	16.4	34	7.8	276
(n = 434)	*S. aureus*	135	31.1	10	2.3	18	4.2	163
	MRSA	94	21.7	9	2.1	13	3.0	116
20-40	CoNS	345	81.4	127	30.0	93	21.9	565
(n = 424)	*S. aureus*	52	12.3	8	1.9	8	1.9	68
	MRSA	42	9.9	7	1.7	5	1.2	54
> 40	CoNS	69	63.3	43	39.5	33	30.3	145
(n = 109)	*S. aureus*	17	15.6	3	2.8	3	2.8	23
	MRSA	12	11.0	2	1.8	0	0.0	14
Total n of samples	CoNS	588	58.8	262	26.2	173	17.3	1,023
(n = 1,000)	*S. aureus*	225	22.5	24	2.4	33	3.3	282
	MRSA	166	16.6	20	2.0	19	1.9	205

CoNS: coagulase negative *Staphylococci*, *S. aureus*: *Staphylococcus aureus*, MRSA: methicillin resistant *Staphylococcus aureus*.

TABLE 2 - Distribution of *Staphylococci* in healthy individuals according to socioeconomic strata.

Socioeconomic status	*Staphylococcal* isolates	Anterior nares n	Anterior nares %	Forearm n	Forearm %	Dorsum and palm n	Dorsum and palm %	Total
Upper economic class	CoNS	155	47.8	85	26.2	59	18.2	299
(n = 324)	*S. aureus*	100	30.9	10	3.1	10	3.1	120
	MRSA	61	18.8	6	1.9	2	0.6	69
Middle economic class	CoNS	158	67.8	67	28.8	40	17.2	265
(n = 233)	*S. aureus*	31	13.3	5	2.2	5	2.2	41
	MRSA	29	12.5	5	2.2	5	2.2	39
Lower economic class	CoNS	134	74.0	40	22.1	28	15.5	202
(n = 181)	*S. aureus*	36	19.9	9	5.0	9	5.0	54
	MRSA	32	17.7	3	1.7	7	3.9	42
Doctors	CoNS	14	51.9	9	33.3	9	33.3	32
(n = 27)	*S. aureus*	11	40.7	1	3.7	3	11.1	15
	MRSA	6	22.2	1	3.7	1	3.7	8
Nurses	CoNS	100	69.4	21	14.6	20	13.9	141
(n = 144)	*S. aureus*	26	18.1	3	2.1	1	0.7	30
	MRSA	23	16.0	3	2.1	1	0.7	27
In-patients	CoNS	27	29.7	15	16.5	17	18.7	59
(n = 91)	*S. aureus*	21	23.1	2	2.2	5	5.5	28
	MRSA	15	6.5	2	2.2	3	3.3	20
Total n of samples	CoNS	588	58.8	262	26.2	173	17.3	1,023
(n = 1,000)	*S. aureus*	225	22.5	24	2.4	33	3.3	282
	MRSA	166	16.6	20	2.0	19	1.9	205

CoNS: coagulase negative *Staphylococci*, *S. aureus*: *Staphylococcus aureus,* MRSA: methicillin resistant *Staphylococcus aureus.*

TABLE 3 - Capsular polysaccharide antigen types in *Staphylococcus aureus* and MRSA isolates as colonizers from healthy individuals.

Organisms	Total n of isolates	Capsular antigen types T5 n	T5 %	T8 n	T8 %	Non T5/T8 n	Non T5/T8 %
Staphylococcus aureus	282	61	21.6	153	54.3	68	24.1
MRSA	205	43	21.0	117	57.1	45	21.9

T5: type 5, T8: type 8, MRSA: methicillin resistant *Staphylococcus aureus.*

DISCUSSION

Resistance to methicillin and the emergence of MRSA has aroused great concerns all over the world during the last few decades. In this work, a 22.5% carrier rate for *S. aureus* and 16.6% for MRSA was determined among the healthy population. These results are consistent with the other studies from India, where 23% to 52.2% *S. aureus* colonization has been reported among healthy individuals[9-12]. Though few studies from India observed no MRSA in the community, a prevalence of 16.6% was determined, which is similar to reports by Saxena et al[9], from Delhi (18%) and Majumdar et al (24%) from Sikkim[9,11]. However, a previous study by Onanuga et al from Nigeria reported 69% of MRSA among healthy women in Zaria, Nigeria[13]. In contrast, studies from the USA, Greece and Malaysia have shown less than 1% colonization of MRSA in healthy individuals[14-16]. Thus MRSA prevalence shows high regional variance. It was quite alarming to note a high incidence of MRSA (54.5%) colonization among children below 5 years of age.

Vancomycin resistance was observed in 1.4% of *S. aureus* isolates, which was confirmed by PCR amplification of *vanA*. Worthy of note was the fact that all the vancomycin resistant strains were resistant to methicillin, except one; ; i.e., one VRSA strain that was sensitive to methicillin was detected, similar to a case reported by Pillai et al[4].

Vancomycin has been the standard of care for serious infections and many studies show 100% positive clinical outcome[16,17]. However, this study determined a 1.8% incidence of vancomycin resistance in the population also presenting MRSA. These findings support the recent increasing emergence of vancomycin-resistant *S. aureus* worldwide[18]. This is also the first report of VRSA in the area studied. Researchers had predicted that the spread of vancomycin resistance was advancing, but the observation of 1.4% resistance in this study confirms the establishment of the resistant strain in this region of southern India. Previous findings concerning high vancomycin-resistant MRSA were reported by Prakash et al in Tamil nadu, southern India[2].

The increased incidence of MRSA and VRSA colonization in the healthy community can be attributed to the indiscriminate use of antibiotics. This is of utmost concern, since *S. aureus* is an opportunistic pathogen that has been implicated as a causative agent in acute food poisoning episodes, toxic shock syndrome, impetigo, scalded skin syndrome, cellulitis, folliculitis and furuncles.

Resistance in MRSA is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin binding protein called PBP2a or PBP21. PBP2a shows decreased affinity for binding beta-lactam antibiotics resulting in resistance not only to methicillin, but to all beta-lactams, including penicillins and cephalosporins[19]. The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate the acquisition of resistance to other antibiotics. Thus, cross-resistance to non-beta-lactam antibiotics, such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin, is common[20].

The presence of type 8 capsular polysaccharide antigen occurred more frequently than type 5 in both the *S. aureus* and MRSA, in agreement with the findings of Paul et al[8].

In conclusion, analysis of the results of this study confirms the need to reassess policies regarding antibiotic use for Staphylococcal infection. Injudicious use of antibiotics will lead to development of multiple drug resistance. Furthermore, regular surveillance of hospital associated infections, including monitoring antibiotic sensitivity pattern of MRSA, and the formulation of a definitive antibiotic policy may be helpful in reducing the incidence of MRSA infection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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