ORIGINAL RESEARCH

Methicillin-resistant *S. aureus* colonization in Mexican children attending day care centres

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Abstract

Purpose: To determine-MRSA carriage rates and genetic relationships of *S. aureus* strains in children attending day care centres in 14 cities from three geographic regions in Mexico.

Materials and methods: Cross-Sectional Study performed in apparently healthy children aged from 6 mo to 6 yr attending day care centres (DCCs). From September 2002 To January 2003, 2345 nasopharyngeal specimens from a similar number of children were collected. Nasopharyngeal samples for bacterial isolation were obtained by standard methods. Antimicrobial susceptibility was determined and genetic relatedness of all MRSA isolates was determined by pulsed field gel electrophoresis (PFGE).

Results: *S. aureus* was identified in 237 children (10.1%), twenty-two children had MRSA for an overall prevalence of MRSA carriage of 0.93%. Children attending DCCs from cities located in the north and south of Mexico showed higher prevalence than children from DCCs in the central region; 1.75%, and 1.71 vs. 0.08%, respectively ($P<0.05$). PFGE demonstrated six different restriction profiles of MRSA with a predominant pattern.

Conclusions: We documented the presence of MRSA strain colonizing children attending DCCs in Mexico, mainly in the south and north regions of the country. Clone

A and B which are closely related represented 45 % of the total of MRSA isolates. We found both, SCCmec type II and type IV strains in the three regions.

Staphylococcus aureus causes a wide variety of infections with considerable morbidity and mortality in normal and immunocompromised hosts.¹ Historically, methicillin-resistant *S. aureus* (MRSA) infections have been linked to health care attention centers.² However, in the last decade, MRSA strains have been found increasingly in the community and they are now an established pathogen in many localities.³ Bacterial genetic studies have found that community-acquired methicillin resistant *S. aureus* (CA-MRSA) has major differences with strains isolated in the hospital setting.⁴

Nasal carriage of *S. aureus* plays a role in respiratory tract infections of critically-ill patients,⁵ and in nosocomial infection in which nasal carriers can be implicated as a source of infection.⁶ Even though the

precise role of nasal carriage of MRSA in healthy people in the community setting is unknown, several studies of *S. aureus* colonization have been carried out in children of communities with high prevalence of CA-MRSA,^{7,8,9} to estimate the prevalence and potential for spread of MRSA in the community. These surveys have documented colonization rates between 1.1 and 61%.^{7,9} The higher rates were found in communities that have reported high prevalence of CA-MRSA infections in children.¹⁰ In Mexico there are no reports of CA-MRSA infections in children, so we do not know if CA-MRSA is not circulating in the community or if physicians are unaware of this threat and are not performing cultures in children with infection in which *S. aureus* plays a role. Children attending DCCs play an important role in the spread of antibiotic-resistant bacteria both, within the facility and from the facility to the community.^{11,12} In this study we looked for MRSA carriage in nasopharyngeal samples of children attending DCCs in some cities of three geographical regions in Mexico. We also determined the antibiotic susceptibility and the genetic profile of the strains.

Materials and methods

The study was approved by the institutional review board of HIMFG. Informed consent was obtained from the parents of each participating child.

Study Design

Cross-sectional study performed by Bacteriology Laboratory at Hospital Infantil de México Federico Gomez. (HIMFG). Nasopharyngeal samples were taken from apparently healthy children from 6 months to 6 years old attending DCCs in cities located in three geographical regions of Mexico. Subjects were enrolled between September 2002 and January 2003. A nominal list of eligible subjects was obtained in each centre and a random selection of children was performed according to a list of computer generated random numbers. Patients who refused to participate

were replaced using the same nominal list and random procedure. The number of children selected in each centre was proportional to the number of children in that centre. Fourteen cities: 6 in the north, (Tecate, Mexicali and Ensenada BC., Tampico, Cd. Madero Tam, and Monterrey. NL.) 6 in the centre (Leon, Gto., San Luis Potosi, SLP., México, DF, Toluca, Edo Mex., Pachuca, Hgo., and Zamora, Mich) and 2 in the south of Mexico (Oaxaca, Oax., and Jalapa Ver) participated in the study. Children with underlying illness or an episode of upper or lower respiratory tract infection in the previous 2 weeks were excluded.

Specimen collection and culture

Nasopharyngeal cultures were obtained with a flexible calcium alginate fibre tipped aluminum applicator swab, (Fisher HealthCare, Hosuton,Tx) which was introduced into the nostrils and advanced until resistance was felt. Swabs were inoculated into Stewart transport medium (Culture swab transport system Spectrum Laboratories, Houston, TX) and processed at the bacteriology laboratory of the HIMFG. Swabs were plated on 5% sheep blood agar (Becton Dickinson Microbiology Systems, Maryland, MD) and incubated overnight at 37°C. Colonies growing on blood agar were identified as *S. aureus* by their typical morphology, grown in 15% of NaCl brain-heart broth (Becton Dickinson Microbiology Systems, Maryland, MD) and biochemical tests (coagulase activity, production of catalase and a positive result in the oxidase test). One colony per plate was then subcultured, harvested and kept frozen at -70° C for further testing.

The minimal inhibitory concentration (MIC) of all isolates was determined by the broth dilution susceptibility test according to the Clinical and Laboratory Standards Institute (CLSI) recommendations.¹³ The antimicrobial agents tested were: oxacillin, amoxicillin, amoxicillin-clavulanate, ticarcillin-clavulanate, cefalotine, cefepime, ceftriaxone, imipenem, erythromycin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, teicoplanin, vancomycin, and linezolid.

TABLE 1. Nasopharyngeal carriage of *S aureus* and of MRSA in children attending DCCs in three different geographic regions of Mexico.

Regions	# DCCs	# children	<i>S. aureus</i>	^a MRSA
Northern states	6	912	111 (12.17%)	16 (1.75 %)
Central states	6	1141	93 (8.15%)	1 (0.08%)
Southern states	2	292	33 (11.3%)	5 (1.71%)
Total	14	2345	237 (10.10)	22 (0.93%)

^a *P* values were 0.00009 and 0.001 between north and south states vs central states respectively.

For all isolates that were erythromycin-resistant and clindamycin susceptible, detection of the presence of inducible macrolide-lincosamide-streptogramin B (MLSB) resistance was performed by disk diffusion with the use of clindamycin and erythromycin disks set 15 to 20 mm apart. Zone shapes were read after a 24-h incubation period at 35°C. A flattened or blunted (“D-shaped”) zone of inhibition around the clindamycin disk on the side facing the erythromycin disk indicated an inducible MLSB-resistant phenotype.¹⁴ SCCmec typing was performed as described Zhang et al.¹⁵ Pulsed-field gel electrophoresis (PFGE) was used to characterize the MRSA isolates as described by McDougall et al.¹⁶

Statistical analysis

Statistical analysis was performed with STATA Analysis version 9.2. College station Texas USA. Data were expressed as mean $\pm$ SD or percentages for each group. The chi-square test with Yates’ correction was used for comparing percentages. Similarities among MRSA isolates were calculated using the Dice coefficient, and a dendrogram of the relationships among MRSA isolates was performed. The robustness of the dendrogram was estimated by cophenetic correlation coefficient (CCC_r) using the Mantel non-parametric test.¹⁸⁻¹⁹

Results

A total of 2,345 NC were collected from a similar number of children. 1141, 912 and 292 children were from cities in the centre, north and south of Mexico

respectively. Age ranged from 6 mo to 6 yr, (mean $\pm$ SD; 4.3 $\pm$ 0.96 yr). No differences were observed in the age from children with MSSA and MRSA (4.56 $\pm$ 0.38 vs. 4.45 $\pm$ 0.97 yr) or among children from the three geographic regions (4.5 $\pm$ 0.9, 4.36 $\pm$ 1.1 and 4.47 $\pm$ 0.87 yr, respectively). Fifty-one percent of the subjects were male. Two-hundred and thirty-seven of the 2,345 sampled children (10.10%) carried *S. aureus* in their nasopharynx. Twenty two of the 2,345 children (0.93%) had MRSA isolates. Rates of MRSA colonization were different between north and south regions compared with central region (*P* = 0.00009 and 0.001 respectively) Table 1.

Susceptibility to antimicrobial agents

The resistance rates of MRSA to erythromycin, clindamycin, trimetoprim/sulfamethoxazol, gentamicin and ciprofloxacin were 72, 32, 22.7, 18.1 and 4.5 % respectively. Two isolates had the inducible MLSB-resistant phenotype. No strain was resistant to vancomycin or linezolid. Thirteen strains had the staphylococcal cassette chromosome mec (SCCmec) type II and nine were type IVa. Isolates with SCCmec type II were resistant to multiple antibiotics, meanwhile type IV isolates were resistant only to erythromycin (Table 2).

Genotype distribution

Among the 22 MRSA strains, six *Sma* I patterns were found, clones A to F (Fig 1). The dendrogram revealed a close relation between clones A and B. Although these clones were found in cities from the three geo-

TABLE 2. Antibiotic resistance profile of MRSA strains according to their SCCmec type.

Antibiotics	Strains with SCCmec type II n=13	Strains with SCCmec type IV n=9
Erythromycin	80%	64%
Clindamycin	64%	0%
Trimetoprim/sulfamethoxazol	22.7%	0%
Gentamicin	36.2%	0%
Ciprofloxacin	8.0%	1%
Vancomycin	0%	0%
Linezolid	0%	0%

graphic areas they were predominant in the north region. Other clones C to F were isolated in children of the three geographic regions. Similarity coefficient ranges of all isolates from DDCs were 56 to 100%.

Discussion

In this study we documented the presence of MRSA strain colonizing children attending DCC in Mexico, mainly in the south and north regions of the country. Clones A and B, which are closely related, represented 45 % of the total MRSA isolates. They were found in

the three regions but predominated in the north of the country.

During the last decade, the epidemiology of *S. aureus* has changed. Currently, in several cities of the United States of America, an important percentage of *S. aureus* isolated from children with community-acquired infections are methicillin-resistant, usually these children lack known risk factors for MRSA infection.²⁰⁻²¹ Studies conducted at sites where CA-MRSA infections are common, have revealed a significant prevalence of MRSA colonization in both

FIGURE 1. Dendrogram of SmaI-PFGE of methicillin-resistant *S. aureus* isolated from children attending DCCs in three geographical regions of Mexico.

children and adults.^{4,7,9} No formal reports about CA-MRSA in children have been published in Mexico, although preliminary data suggest that MRSA has appeared in the community.²²

Ten percent of the children attending Mexican DCCs carried *S. aureus* in the nasopharynx. This figure is lower than at other pediatric centres in the USA. Different studies published after 2000 show that the colonization rate of *S. aureus* in healthy American children is >20%, although some regional variations exist.^{7,9,23} However, Regev-Yochay et al.²⁴, assessing the colonization rate of *S. aureus* in 790 children aged 5 days to 40 months (median, 1.3 yr) in Israel, discovered that 80 children (10%) were *S. aureus* carriers; a finding similar to our results.

We found low prevalence of nasopharyngeal carriage of MRSA in Mexican children attending DCCs (0.93%). This result is similar to the 0.8% found by Nakamura et al, in healthy children living in Nashville, TN, in 2001.²⁵ However, three years later a ten-fold increase in MRSA colonization (0.8% in 2001, 9.2% in 2004; $P < 0.001$) was found in the same institution.⁹ These findings suggest that, when the cases of community MRSA infection begin to appear, as in Mexico, the prevalence of MRSA carriers in healthy children is low. But, shortly thereafter, a remarkable increase in the MRSA colonization rate can be observed.

There were no differences in the rate of colonization of *S. aureus* in children attending DCCs in the northern, central or southern states of Mexico (12.1% vs. 8.15% vs. 11.3%, respectively). However, the prevalence of methicillin resistance in children attending DCCs in the northern and southern states was higher than in those attending DCCs in the central part of Mexico. What could explain this difference in the prevalence of MRSA carriage?

In addition to geographic variation, the movement of people across the border may explain the higher rates of MRSA colonization in children living in border cities or in southern cities with high rates of mi-

gration to American cities where high rates of MRSA colonization or infections have been documented.¹²

A striking finding was the presence of isolates with SCCmec type II, its PFGE profile has similarity with USA 100 clone suggesting a health care associated source for these strain.¹⁶ Unfortunately, no information was collected about MRSA risk factors, so we only can suppose that this particular clone has found an appropriate niche in this population, perhaps favoured by migration movements, by people going in and out of hospital and/or by genetic adaptation of the bacteria. Confirmation of this hypothesis is outside the scope of this study but deserves further exploration. One more anticipated finding was the presence of SCCmec type IV strains with several genetic backgrounds which is in accordance with the initial reports of the emergence of MRSA in the community.¹⁷ After these initial findings the emergence of a predominant clone (USA 300) widely disseminated across the country occurred in the USA.²⁶ We hypothesize that, in the years following this study, a clone maybe USA 300 will be found among MRSA isolates of the community in Mexico.

Our study has limitations. It was preformed over five months (autumn and winter) and, thus, it was not possible to assess seasonal variation in colonization rate. Although the study included three geographical areas in Mexico, not all the existing DCCs (or those randomly selected) in each region were included. Therefore, these results only provide an estimation of the colonization rates and do not reflect the actual rates throughout the country. Despite this limitation we have documented for the first time the presence of MRSA in the country highlighting the need to be alert of this risk and maintain a continuous surveillance.

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