Epidemiologic Trends of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Among Non-Institutionalized Pediatric Populace of Iligan City

MARIE JOHANNA L. CUADRA
LUCILYN D. LAHOYLAHOY

Abstract

Nasal surveillance swab cultures of one hundred fourteen pediatric subjects (70 day care attendees and 44 pediatric outpatients) of Iligan City were gathered from April to December 2006 to determine staphylococcal colonization rates. 7Morphological and biochemical methods were employed for the characterization and presumptive identification of 327 strains that were isolated from the primary culture medium, mannitol salt agar (MSA). All recovered isolates were Gram positive staphylococci and affirmative of catalase reaction. Mannitol fermenting isolates corresponded to the number of strains which exhibited positive coagulase reaction and were presumptively identified as *Staphylococcus aureus* while the remaining non-mannitol fermenters were designated as coagulase-negative staphylococci. Seventy-three children harbored *S. aureus* in their anterior nares whereas 70 were colonized with CoNS. However, there is no significant correlation between *S. aureus* and CoNS colonization among the risk factors considered in the study ($p>0.05$).

MARIE JOHANNA L. CUADRA, B.S. Biology Graduate, Department of Biological Sciences, College of Science & Mathematics, MSU-IIT, LUCILYN D. LAHOYLAHOY, Professor, Department of Biological Sciences, College of Science & Mathematics, MSU-IIT.

Non-duplicate *S. aureus* (73) and coagulase-negative staphylococci (70) were tested for their susceptibility to oxacillin (1μg), cefoxitin (30μg), vancomycin (30μg), erythromycin (15μg), and clindamycin (2μg). Susceptibility to vancomycin was high among *S. aureus* strains (82%) whereas among coagulase-negative staphylococci posed great sensitivity to cefoxitin (60%). The least effective drug in the eradication of *S. aureus* strains was erythromycin with resistance rate of 41% while clindamycin revealed high number of resistance strains (53%) among CoNS. Twenty-one strains were oxacillin-resistant *S. aureus* however, only 13 of these were identified as true MRSA strains which exhibited resistance to both cefoxitin and oxacillin which were found among children with no contact among healthcare workers. Four MRSA strains were detected to be vancomycin-resistant as well. Resistance of MRSA to two or more antimicrobials was designated as multidrug resistant MRSA (MDR-MRSA, n=7) in which five MRSA strains have high resistance to both erythromycin and clindamycin (38%) and two strains resistant to erythromycin, clindamycin and vancomycin. There is no significant correlation between the susceptibility patterns and risk factors considered among the study populace ($p>0.05$).

The ecological niche of *Staphylococcus aureus* in the anterior nares and asymptomatic colonization of 25-40% on the general population is far more common than infection. The pediatric populace tends to have higher colonization rates (approximately more than 40%) due to their frequent contact with respiratory secretions and incidences of childhood illnesses (Paediatric and Child Health, 1999; Chambers, 2001). Asymptomatic colonization represents a reservoir for subsequent staphylococcal infections, which is now considered to be of serious concern due to the increasing incidence of staphylococcal resistance to most of the commercially available antibiotics (Oxoid Limited, 2001).

Shortly after the introduction of methicillin in 1960, strains of methicillin-resistant *S. aureus* (MRSA) were detected in a hospital setting in United Kingdom (1961) and has spread nosocomially around the world (Masterton, 2002). It is one of the most widespread, multiple drug resistant, virulent nosocomial pathogens, making infections difficult to treat (Cooper *et al.*, 2004). Moreover, this hospital-acquired MRSA (HA-MRSA) accounts for 30-70% of all nosocomial *S. aureus* isolates.

Alarmingly, the situation changed rather dramatically of which MRSA is no longer regarded as a strictly nosocomial pathogen and a disturbing increase have developed and spread outside hospital settings worldwide (Maltezou and

Giamarellou, 2006). Furthermore, the prevalence of MRSA colonization and infection has escalated among individuals who lack traditional risk factors. This variant of MRSA, the community-associated methicillin-resistant *S. aureus* (CA-MRSA, 2006), accounts for 35-50% of staphylococcal isolates in various geographic areas worldwide and is spread by direct physical contact, indirect contact and living in crowded settings. Unlike hospital strains, CA-MRSA strains have discrete microbiologic and genetic properties, tend to cause different types of infections and differ in typical antibiotic resistance profiles (Harris, 2006; MRSA, 2006).

The number of populations at risk for CA-MRSA is reported to be steadily expanding with high infectivity among children. Detection of CA-MRSA among asymptomatic children with no identifiable precedence for colonization and infection indicates that sustained transmission was occurring in the community and is emerging as an important outpatient pathogen (Chambers, 2001). However, the epidemiology of MRSA in outpatient settings, where most MRSA-related infections are being treated and the greatest percentage of antimicrobial use occurs, has not been fully investigated to date in the Philippines. The detection of nasal colonization of CA-MRSA in children is of great clinical relevance as it antedates bacteremic as well as non-bacteremic infections (Gray, 2004; Singh *et al.*, 2003).

Objectives of the Study

This study was undertaken to establish representative community surveillance estimates of community-associated MRSA carriage among non-institutionalized pediatric population of Iligan City.

Specifically, this aims to:

1. Conduct preliminary assessment on the MRSA colonization rates among non-institutionalized children of Iligan City;
2. Evaluate risk factors associated with MRSA colonization: hospitalization, history of antibiotic use, and current illness; and
3. Determine and construct community-associated MRSA antibiograms.

Materials and Methods

Study Population. The individuals enrolled in the study were children ages 3 to 6 years old who were day care attendees of selected municipal day care centers in

Iligan City and those who were seeking medical attention on local outpatient clinics.

Collection of Nasal Swabs. Nasal surveillance swab specimens were collected using dry sterile cotton swabs. Specimens were placed on properly labeled screw cap tubes with Amies Transport Medium.

Detection, purification and maintenance of isolates from nasal swab specimens. Swab specimens were plated on the primary culture medium, mannitol salt agar (MSA) for the isolation of staphylococci. Incubation of plates was made for a maximum of 120 hours at room temperature. Presumed staphylococcal colonies were characterized as mannitol fermenters and non mannitol fermenters. Representative colonies were purified, and stocked on nutrient broth to ensure viability.

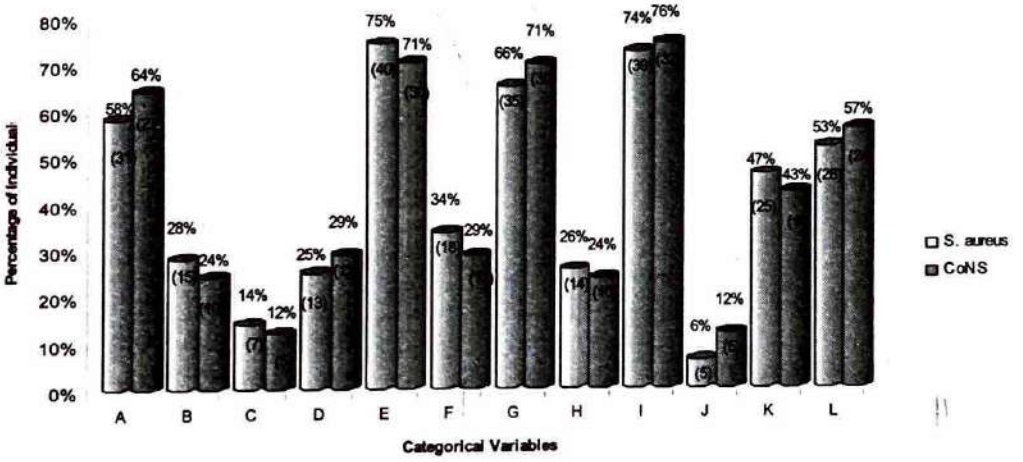
Morphological and Biochemical Methods. Bacterial strains were morphologically examined through employment of Gram staining technique and microscopy. Twenty-four hour old strains were biochemically tested by catalase and coagulase tests. Coagulase-positive strains were identified as *S. aureus* otherwise, isolates were classified as coagulase-negative staphylococci (CoNS).

Antibiotic Susceptibility Testing. The Kirby Bauer Disc Diffusion method was employed for the antibiograms or antibiotic susceptibility patterns of identified staphylococcal isolates against the following antistaphylococcal agents: oxacillin (1μg), cefoxitin (30μg), vancomycin (30μg), erythromycin (15μg) and clindamycin (2μg). Following the 0.5 MacFarland Turbidity Standard, bacterial suspensions of non duplicate *S. aureus* and CoNS isolates were prepared and swab streaked onto properly labeled Mueller Hinton Agar plates. Oxacillin, cefoxitin, and vancomycin (excluded in CoNS) discs were aseptically placed on the agar surface 26mm apart while erythromycin and clindamycin were separately placed 15mm to detect inducible macrolide-lincosamide-streptogramin phenotypes in isolates that are susceptible to clindamycin and resistant to erythromycin (D-Test).

Statistical Analyses. Categorical comparisons were performed using Chi-square analyses and Fisher's Exact Test. A p value of < 0.05 was considered significant for all comparisons. Risk factors for MRSA colonization were evaluated by using Fisher's Exact Test and antibiotic resistant profiles were compared using chi-square test. Variables achieving $p < 0.05$ in the final model were considered significant, and odd ratios (Ors) with 95% confidence intervals (CIs) were calculated.

Results and Discussion

One hundred fourteen pediatric respondents underwent the same nasal specimen acquisition procedure and yielded three hundred twenty seven presumed staphylococcal colonies, all Gram positive and affirmative of catalytic activity. One hundred eighteen mannitol fermenting isolates were found to be coagulase positive strains and were presumptively identified as *S. aureus* whereas two hundred nine isolates were classified as coagulase negative staphylococci (CoNS). The colonization rates of day care subgroup are shown in Figure 1.



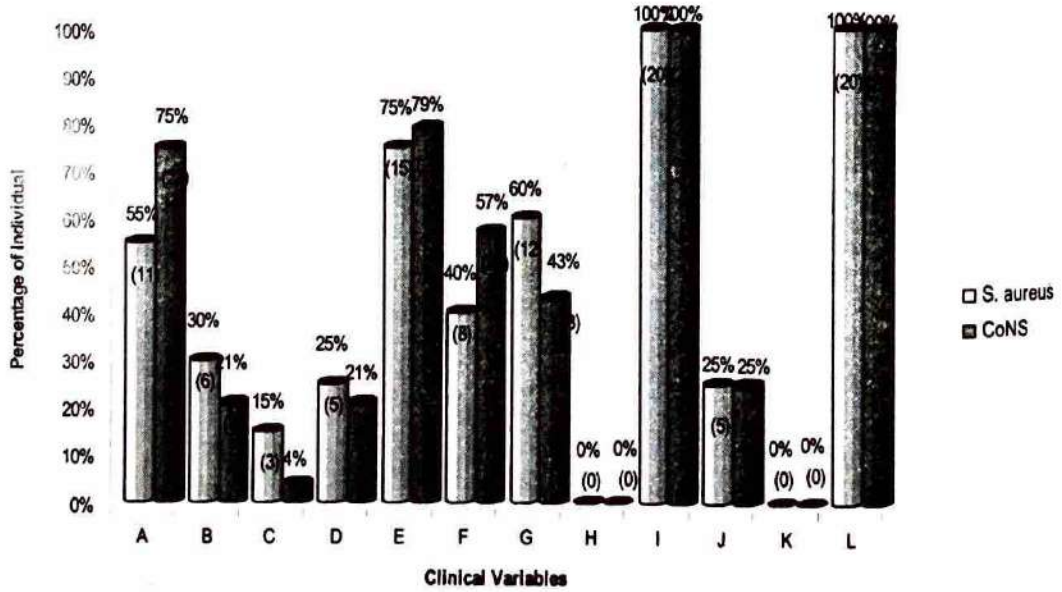
Legend:
A=antibiotic user
B=non antibiotic user
C=herbal medicine user
D=with history of hospitalization
E=without history of hospitalization
F=previous contact among healthcare workers
G=no previous contact among healthcare workers
H=with contact among hospitalized individuals
I=without contact among hospitalized individuals
J=exposure to cigarette smoke
K=asymptomatic
L=symptomatic

Figure 1. Colonization rates of *S. aureus* and CoNS among day care attendees in correlation with identified categorical variables.

Of the seventy day care attendees, fifty-three subjects were colonized with *S. aureus* whereas there were forty-two subjects who were colonized with CoNS. There were day care students who were experiencing common childhood illnesses at time of sampling and were noted to have visible nasal secretions.

High staphylococcal colonization was later observed (*S. aureus* 53%, CoNS 57%). Symptomatic individuals are designated to have higher bacterial inhabitation rates as well as of greater risk for acquisition of potentially pathogenic microorganisms because of its suitable moist environment for bacterial proliferation and the respondents' compromised immune system (Butwin, 2004). Most staphylococcal strains were reported to be of nosocomial derivation with healthcare or hospital workers being commented as potential carriers and culprits of staphylococcal diseases (Florentino, 2000). However, high staphylococcal colonization rates were still detected (*S. aureus* 75%, CoNS 71%) from students who did not have any history of hospital admittance.

Whereas, among the forty-four recruited pediatric patients, there were 20 individuals who were nasal carriers *S. aureus* while 28 were colonized with CoNS. Pediatric outpatients who had no contact among hospitalized individuals have high colonization of *S. aureus* and CoNS (100%) as well as those without history of hospitalization (SA 75% and CoNS 79%) (Figure 2).



Legend:
A=antibiotic user
B=non antibiotic user
C=herbal medicine user
D=with history of hospitalization
E=without history of hospitalization
F=previous contact among healthcare workers
G=no previous contact among healthcare workers
H=with contact among hospitalized individuals
I=without contact among hospitalized individuals
J=exposure to cigarette smoke
K=asymptomatic
L=symptomatic

Figure 1. Colonization rates of *S. aureus* and CoNS among pediatric outpatients in correlation with identified categorical variables.

Colonization might be mediated by some factors that were not tackled in the study such as fomites and other indirect modes of transmission. Coagulase-negative staphylococcal-colonization among antibiotic users was substantially high at 73%. Lower colonization rate was observed among other clinical factors, however, there are no significant differences between colonization of *S. aureus* or CoNS and the clinical factors considered ($p > 0.05$).

Antibiotic Susceptibility Patterns of *S. aureus*. Antimicrobial resistance is a growing problem worldwide with serious clinical implications. The dramatic reduction of therapeutic options to treat patients infected with this microorganism is of great concern. Duplicate isolates were excluded and a total of 73 presumptively identified *S. aureus* strains were subjected to Kirby-Bauer disc diffusion test for determination of antibiograms against antistaphylococcal agents such as oxacillin (1μg), cefoxitin (30μg), vancomycin (30μg), erythromycin (15μg) and clindamycin (2μg). All *S. aureus* isolates exhibited positive growth in Mueller-Hinton agar. Zones of inhibition were measured to the nearest millimeter after 18 hours of incubation at room temperature and were correspondingly interpreted as susceptible (S), susceptible-dose dependent (SDD) or resistant (R).

Among the five drugs employed, resistance to erythromycin was exhibited highly (41%) by *S. aureus* strains. Vancomycin revealed the greatest rate (82%) of non-resistance by the strains thus designating this drug as the most efficient in inhibiting staphylococcal growth as opposed to other antimicrobials tested in this study. Twenty-six percent of the strains fell under clindamycin susceptible-dose dependent criteria, which implied a possible resistance by strains at forthcoming time to this antimicrobial agent. Figure 3 shows the interpretative trend of susceptibility of *S. aureus* strain against the antistaphylococcal agents.

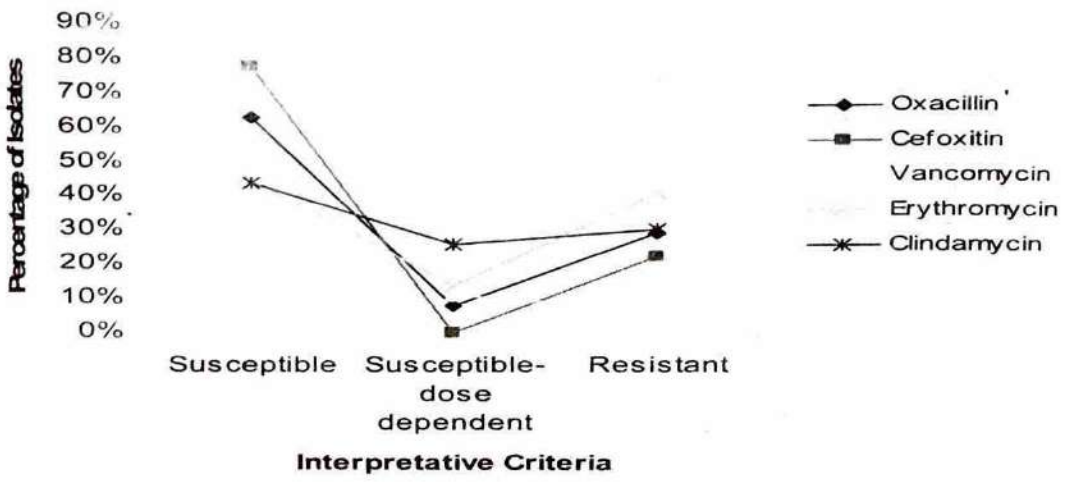


Figure 3. Phenotypic susceptibility characterization of *S. aureus* isolates against antibiotics.

Detection of Methicillin-Resistant and Oxacillin-Resistant *Staphylococcus aureus*. Recent MRSA studies have relied on testing disc diffusion assay using oxacillin screening alone. However, possible errors may exist in the interpretation of methicillin resistance among isolates. Cefoxitin, in this study, was employed to detect heteroresistant strains and confirmation of the presence of *mec* A gene that encodes for methicillin resistance among staphylococci. Table 1 shows the antimicrobial combination for the detection of MRSA and ORSA strains.

Twenty-one *S. aureus* isolates were detected to be resistant to oxacillin, however, only 13 of these were confirmed to have resistance to both oxacillin and cefoxitin and are designated as true methicillin-resistant *S. aureus* (MRSA) whereas the remaining isolates (8) exhibited resistance to oxacillin but were still sensitive to cefoxitin were classified as oxacilliin-resistant *S. aureus* (ORSA).

Table 1. Methicillin resistance among *Staphylococcus aureus* correlated with categorical variables.

CLINICAL VARIABLES	ANTIBIOTIC COMBINATION					
	OX^{S}-FOX^{S}	OX^{SDD}-FOX^{S}	OX^{S}-FOX^{R}	OX^{SDD}-FOX^{R}	OX^{R}-FOX^{R}	OX^{R}-FOX^{S}
A	29	4	1	0	8	4
B	6	1	0	0	2	3
C	8	0	1	0	3	1
D	7	2	1	0	4	2
E	36	3	1	0	9	6
F	18	1	1	0	2	4
G	25	4	1	0	11	3
H	7	0	1	0	3	1
I	36	5	1	0	10	7
J	8	4	0	0	2	2
K	14	3	2	0	4	3
L	29	2	0	0	9	5

Legend:
A=antibiotic user
B=non antibiotic user
C=herbal medicine user
D=with history of hospitalization
E=without history of hospitalization
F=previous contact among healthcare workers
G=no previous contact among healthcare workers
H=with contact among hospitalized individuals
I=without contact among hospitalized individuals
J=exposure to cigarette smoke
K=asymptomatic
L=symptomatic
S=susceptible
SDD=susceptible-dose dependent
R=resistant

The eight true ORSA strains were detected among those who had no contact among hospitalized individuals (5 from day care attendees and 3 from outpatients). Of the thirteen identified true MRSA, 8 (62%) of these were carried by day care attendees while the remaining strains (5, 38%) were from pediatric outpatients. These MRSA strains were prevalent in the children populace with no contact among healthcare workers prior to the study. However, colonization of MRSA has no significant correlation among the said variable ($p > 0.05$). This could be due to the notion of lacking association between the prevalence of MRSA in the pediatric populace and with community-associated MRSA being not yet a known epidemic in the locality of Iligan as well as in the Philippines.

Multiple Drug Resistant Methicillin-Resistant *Staphylococcus aureus*. Methicillin-resistant *S. aureus* strains have assumed increasing importance internationally as a cause of both nosocomial and community-acquired infections. Until recently, most MRSA strains were multidrug resistant, nosocomially acquired, and prone to become endemic in hospitals. Community-acquired strains of MRSA, with few or no additional resistances apart from ß-lactams, have been described only quite recently from a number of countries, including Australia, New Zealand, Canada, the United States, and Saudi Arabia (Bell and Turnidge, 2002). Non-susceptibility to at least three of the employed antibiotics (erythromycin, clindamycin and vancomycin) was described as a multidrug-resistant strain (Table 2).

Table 2. Multiple-resistance of methicillin resistant *S. aureus* to other antimicrobials

CLINICAL VARIABLES	ANTIBIOTIC COMBINATIONS ON MULTI-DRUG RESISTANT MRSA			
	ERY^R-DA^R	ERY^R-V^R	DA^R-V^R	ERY^R-DA^R-V^R
A	4	2	2	1
B	1	0	0	0
C	0	2	1	1
D	1	0	0	0
E	4	4	3	2
F	0	0	0	0
G	5	4	3	2
H	1	0	0	0
I	5	4	3	2
J	3	0	0	1
K	2	0	0	0
L	4	4	3	2

Legend:
A=antibiotic user
B=non antibiotic user
C=herbal medicine user
D=with history of hospitalization
E=without history of hospitalization
F=previous contact among healthcare workers
G=no previous contact among healthcare workers
H=with contact among hospitalized individuals
I=without contact among hospitalized individuals
J=exposure to cigarette smoke
K=asymptomatic
L=symptomatic

Of the 13 confirmed MRSA strains in this investigation, seven of these were detected to gain multiple resistances to any of the antimicrobials. Five MRSA strains exhibited resistance to both erythromycin-clindamycin resistance and were isolated from children who had no contact among healthcare workers and without history of hospitalization while non-susceptibility of strains to erythromycin and vancomycin (4/7) was recovered from children who had no contact among hospitalized individuals, without history of hospitalization, without contact among healthcare workers and among symptomatic subjects. With the same risk factors mentioned, only two strains of MRSA recovered from revealed resistance to erythromycin, clindamycin and vancomycin.

Multidrug-resistant methicillin-sensitive *S. aureus* (MSSA) was also noted in this study only that fourteen strains exhibited non-susceptibility to both erythromycin and clindamycin. Such characteristic was recovered from individuals with no established risk factors.

Conclusion and Recommendation

In this study, CA-MRSA was detected among the pediatric populace though found in lower rates, detection was still affirmative among the bacterial isolates harbored by this group. Rates of MRSA and MDR-MRSA vary worldwide and in different populace however, the 15% (11/73) MRSA and 54% MDR-MRSA carriage rate still pose a considerable concern since treatment failure is possible in the eradication of infections caused by *S. aureus*.

This investigation highly advocates the necessity of a more widespread surveillance study and acquiring more pediatric subjects on the evaluation of the nature of *S. aureus* in terms of resistance to methicillin/oxacillin. A longitudinal study would also be of great relevance to determine *S. aureus* colonization and the factors associated with it so that the bacteria's origin would be determined whether these were community-associated or hospital-acquired. Evaluating the potential risk factors associated in *S. aureus* is of great relevance to investigate its source and would decrease the emergence of maladies caused by this pathogen. There is a need also to study at molecular level to establish the classification of the gene that confirms methicillin resistance among staphylococcal strains through employment of the polymerase chain reaction or PCR.

Literature Cited

Bell and Turnidge. 2002. Antimicrobial resistance trends in community-acquired respiratory tract pathogens in the Western Pacific Region and South Africa: report from the SENTRY antimicrobial surveillance program. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve

Butwin, J. 2004. Indiana State Department of Health Guidelines for Methicillin-Resistant *Staphylococcus aureus* in Indiana Schools. http://64.233.167.104/search?q=cache:1sMHkEMv8ScJ:www.in.gov/isdh/dataandstats/epidem/2004/sep/guidelines.pdf+MRSA+Butwin&hl=en&ct=clnk&cd=

CA-MRSA Fact Sheet. 2006. http://www.southernnevadahealthdistrict.org/download/disease_factsheets/ca-mrsa_patient_facts_05.pdf.

CDC Issues. 2000. http://www.cdc.gov/ncidod/eid/vol7no3_supp/naimi.htm. Date Accessed: CDC Antimicrobial Surveillance. 2005. http://www.cdc.gov/drugresistance/surveillance.htm

Chambers, H. 2001. Special Issue: The Changing Epidemiology of Staphylococcus aureus?

Chavez-Bueno *et al.* 2005. Inducible Clindamycin Resistance and Molecular Epidemiologic Trends of Pediatric Community-Acquired Methicillin-Resistant Staphylococcus aureus in Dallas, Texas. Antimicrobial Agents and Chemotherapy.

Creech *et al.* 2004. Increasing rates of nasal carriage of methicillin-resistant Staphylococcus aureus in healthy children. Pediatric Infectious Disease Journal. Date Accessed:

Dale and Mandelstam. 2005. Antibiotics. Microsoft® Encarta® 2006 [CD ROM]. Redmond, WA: Microsoft Corporation, 2005.

Darini and Palazzo, 2004. Cefoxitin does not induce production of penicillin binding protein 2a in methicillin-susceptible Staphylococcus aureus strains. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=PubMed&list_uids=1536&dopt=Abstract

de Brito *et al.* 2006. Outbreaks Associated To Bloodstream Infections With *Staphylococcus aureus* and Coagulase-Negative *Staphylococcus Spp* In Premature Neonates In A University Hospital From Brazil. http://64.233.167.104/search?q=cache:X26z4ul2jYEJ:www.scielo.br/pdf/bjm/v37n2/arq01.pdf+de+Brito+et+al.+2006+staphylococci&hl=en&ct=clnk&cd=2&gl=ph

Denis *et al.* 2002. Microbiological surveillance of methicillin-resistant *Staphylococcus aureus* in Belgian hospitals : 1992 -2002. http://www.belgianinfectioncontrolsociety.be/pdf/MRSA2003/webdenis.pdf

Drew *et al.* 2006. Emerging Options for Treatment of Invasive, Multidrug-Resistant Staphylococcus aureus Infections. Pharmacotherapy. http://lib.bioinfo.pl/auth:Drew,RH

Fridkin *et al.* 2006. Methicillin-Resistant Staphylococcus aureus Disease in Three Communities. The New England Journal of Medicine

Fiebelkorn *et. al.* 2003. Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci. http://jcm.asm.org/cgi/content/abstract/41/10/4740

Florentino, Paul. 2000. Antibiotic Resistance is a Global Threat Return to: American College of Physicians-American Society of Internal Medicine Annual Session 2000.

Fokas *et. al.* 2005. Prevalence of inducible clindamycin resistance in macrolide-resistant *Staphylococcus* spp. http://www.blackwell-synergy.com/links/doi/10.1111/j.1469-0691.2005.01101.x/abs/

Gadepalli and Dhawan, 2006. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus.

Gosbell *et al.* 2003. Detection of intrinsic oxacillin resistance in non-multiresistant, oxacillin-resistant Staphylococcus aureus (NORSA).

Gray, J. W. 2004. MRSA: the problem reaches paediatrics. Archives of Disease in Childhood. http://adc.bmj.com/cgi/content/full/89/4/297

Grayson, L. 2006. The Treatment Triangle for Staphylococcal Infections. The New England Journal of Medicine

Hanselman et al., 2006. Methicillin-Resistant Staphylococcus aureus Colonization in Veterinary Personnel. Emerging Infectious Disease.

Kaila and Taback. 2001. The Effect of Day Care Exposure on the Risk of Developing Type 1 Diabetes. http:// care. diabetesjournals.org/cgi/reprint/ 24/8/1353.

Harris, A. 2006. MRSA: Emerging Infections. http:// www. Umm .edu/ doctors/ anthony__ harris.html

Lim *et al.* 2002. Prevalence of resistance to macrolide, lincosamide, and streptogramin antibiotics in Gram-positive cocci isolated in a Korean Hospital. Journal of Antimicrobial Therapy

Lowy, FD. 1998. Coagulase Test – The Staphylococci. http:// www. ratsteachmicro.com/Staphylococci_Notes/HCOE_CAI_Review_Notes_Staphylococci.htm

Masterton. 2002. A New Understanding of Antibiotic Resistance in Nosocomial Infections. http://www.infectionacademy.org/expert_reviews/Paterson_Review4.pdf.

Maltezou and Giamarellou. 2006. Community-acquired methicillin-resistant Staphylococcus aureus infections.

MRSA. 2006. http://www.ccohs.ca/oshanswers/biol_hazards/methicillin.html

Oxoid Limited. 2001. Isolating *Staphylococcus Aureus* http://www.oxoid.com/UK/blue/techsupport/its.asp?itsp=feature&page=feature3.

Paediatric and Child Health. 1999.

Redmund. 2005. Pediatrics. . Microsoft® Encarta® 2006 [CD ROM]. Microsoft Corporation, 2005.

Reynolds, J. 2005. Kirby-Bauer Test for Antibiotic Susceptibility. http://www.rlc.dcccd.edu/mathsci/reynolds/micro/lab_manual/antibiotics.html

Singh *et al.* 2003. Microbiologic Surveillance Using Nasal Cultures Alone Is Sufficient for Detection of Methicillin-Resistant Staphylococcus aureus Isolates in Neonates. Journal of Clinical Microbiology

Skov. 2006. Evaluation of a cefoxitin 30 μg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. http://64.233.167.104/search?q=cache:k8FUPvBj9UJ:www.bsac.org.uk/_db/_downloads/The_Use_of_Cefoxitin_for_the_Determination_of.ppt+Skov+oxacillin&hl=en&ct=clnk&cd=1&gl=ph

Spock, B. 2007. ORSA - Oxacillin Resistant Staphylococcus Aureus. http://www.caercoork.com/orsa/orsa.html

Steward *et al.* 2005. Testing for Induction of Clindamycin Resistance in Erythromycin-Resistant Isoaltes of Staphylococcus aureus, Journal of Clinical Microbiology

Todar, K. 2005. *Staphylococcus*. Todar's Online Textbook of Bacteriology. http://www.textbookofbacteriology.net/.

Tolan 2006. *Staphylococcus aureus* Infection. http://www.emedicine.com/PED/topic2704.htm.

Tuvlin, J. 2006. Antibiotic Susceptibility Tests: Interpretation, Predictive Value, and Results. http://www.acponline.org/ear/vas2000/atb.htm

Von Eiff *et al.* 2001. Intracellular Persistence of *Staphylococcus aureus* Small-Colony Variants within Keratinocytes: A Cause for Antibiotic Treatment Failure in a Patient with Darier's Disease.