

Antimicrobial Resistance among Clinical Isolates of *Staphylococcus aureus* in Thailand from 2000 to 2005

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From 2000 to 2005, the data of all clinical isolates of *Staphylococcus aureus* including methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) from 28 hospitals in the National Antimicrobial Resistance Surveillance, Thailand (NARST) program were reviewed and analyzed for the prevalence and pattern of antimicrobial susceptibility by WHONET software program. Among all isolates, around 26% of MRSA have been noted in each year. The rates of erythromycin-resistant MRSA were relatively high, ranging from 94.5% to 96.8%, followed by clindamycin resistant (37.4% to 68.9%), fosfomycin-resistant (7.7% to 17%), vancomycin-resistant (0.1% to 0.8%), and teicoplanin resistant (0.2% to 1.3%). The rates of antimicrobial resistance MSSA were constantly low, with erythromycin resistance ranging from 3.7% to 4.6%, clindamycin resistance ranging from 1.4% to 2.3%, fosfomycin resistance ranging from 0.7% to 1.4%, vancomycin resistance ranging from 0.1% to 1.2%, and teicoplanin resistance ranging from 0.1% to 1.1%. An increasing trend of vancomycin resistance in *S. aureus* determined by the disk diffusion method should be further confirmed by appropriate susceptibility methods. Molecular typing methods are needed to determine the epidemiological association between these resistant isolates.

Keywords: Anti-bacterial agents, Drug resistance, multiple, bacterial, Methicillin resistance, Microbial sensitivity tests, *Staphylococcus aureus*, Thailand

J Med Assoc Thai 2009; 92 (Suppl 4): S8-18

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Staphylococcus aureus is an important cause of healthcare-associated infections around the world and infects both hospitalized patients with decreased host defenses and healthy immunologically competent individuals in the community^(1,2). *S. aureus* is the causative agent of a wide range of diseases from mild infections including carbuncles and food poisoning, through more serious device- and wound-related infections, to life-threatening conditions including bacteremia, necrotizing pneumonia, and endocarditis. *S. aureus* produces a plethora of virulence

factors that facilitate attachment, colonization, cell-cell interactions, immune evasion, and tissue damage⁽³⁾.

Methicillin-susceptible *S. aureus* (MSSA) becomes methicillin-resistant *S. aureus* (MRSA) by the acquisition of the *mecA* gene, which encodes for PBP2a, a penicillin-binding protein, with low-binding affinity to practically all beta-lactam antibiotics currently available⁽⁴⁾. MRSA emerged in the 1980s as a major clinical and epidemiologic problem in hospitals, and has acquired genes encoding proteins involving in resistance to all penicillins, including methicillin and other narrow-spectrum beta-lactamase-resistant penicillin antibiotics⁽⁵⁾. MRSA has spread out of the hospitals and into communities. Infections caused by this organism are emerging as a major threat in the

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community⁽⁶⁾. The National Nosocomial Infections Surveillance System reported that the prevalence of MRSA infection in hospitals increased from 2.1% in 1975 to 35% in 1991⁽⁷⁾. Data from the SENTRY Antimicrobial Surveillance Program collected from 1997 to 1999 revealed that the MRSA prevalence varied as follows: Western Pacific region (46%), the United States (34.2%), Latin America (34.9%), Europe (26.3%), and Canada (5.7%). While in European centers, the prevalence rates varied from less than 2% in the Netherlands to 54.4% in Portugal. In Western Pacific countries, the occurrence of MRSA ranged from 23.6% in Australia to more than 70% in Japan and Hong Kong^(8,9). About 30-60% of *S. aureus* isolates in Thailand were MRSA, a major causative agent of nosocomial infections. The high percentage is probably due to inadequate infection control in hospitals⁽¹⁰⁾.

Vancomycin is the treatment of choice for serious MRSA infections. Unfortunately increased use of vancomycin might lead to the emergence of MRSA strains that are also resistant to vancomycin as well. Since 1997, infections caused by MRSA strains with reduced susceptibility to vancomycin have been reported from Japan⁽¹¹⁾, France⁽¹²⁾, the United States⁽¹³⁾, Korea⁽¹⁴⁾, Germany⁽¹⁵⁾, Belgium⁽¹⁶⁾, and Thailand⁽¹⁷⁾. The minimal inhibitory concentration (MIC) for vancomycin of these strains was 8 µg/ml, and vancomycin therapy was unsuccessful in all of these infected patients. While in the past, most serious *S. aureus* infections were successfully treated with a certain type of antibiotic related to penicillin, the therapy for *S. aureus* infections has now become more problematic than ever. Prevention is now very essential.

The primary objective of this study was to describe the results of the National Antimicrobial Resistance Surveillance Thailand (NARST) on clinical isolates of *S. aureus* collected from 28 hospitals from 2000 to 2005.

Material and Method

Bacterial isolates

Since 1998, NARST, of the Department of Medical Sciences, has been organized for investigations of antimicrobial resistance of various pathogenic bacteria in Thailand. The program was technically supported by the World Health Organization. Activities of the program include strengthening the standardization of microbiology laboratory practice in identification of bacteria and antimicrobial susceptibility testing.

During the period between January 1, 2000 and December 31, 2005, all clinical isolates of *S. aureus* were recovered in the clinical microbiology laboratories of 28 hospitals in different parts of Thailand (5 in the North, 6 in the Northeast, 9 in the Central, 4 in the East, and 4 in the South). All isolates were selected for surveillance of antimicrobial resistance and data analysis using WHONET software program. Isolation and identification of *S. aureus* were performed using the conventional cultures and biochemical methods at the hospital laboratories according to the standard guidelines. All data from each hospital including type of specimen, bacterial isolation, and diameter of antimicrobial inhibition zone were sent to the NARST center (Nonthaburi, Thailand).

Antimicrobial susceptibility tests

Susceptibility to oxacillin, erythromycin, clindamycin, vancomycin, teicoplanin, and fosfomycin was determined by the disk diffusion method according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) [formerly National Committee for Clinical Laboratory Standards (NCCLS)]⁽¹⁸⁾. The interpretation for the inhibition zone size was based on the interpretive standards for *S. aureus*, of the CLSI.

Statistical analysis

The prevalence of *S. aureus* isolates, clinical epidemiology, and antimicrobial susceptibility during 2000 and 2005 were collected and analyzed using WHONET software program. Multiple isolates from different sites of a single patient were counted only once and antimicrobial susceptibility determination of the first isolate was used in this study. Descriptive statistics was presented in terms of number and percentage.

Results

A total of 11,640, 12,131, 12,468, 12,288, 13,053, and 12,268 isolates of *S. aureus* were collected in 2000, 2001, 2002, 2003, 2004, and 2005, respectively. Among these *S. aureus* isolates, around 24% to 27% of MRSA was noted in each year (Fig. 1).

Rates of antimicrobial resistance of MRSA and MSSA isolates (Fig. 2)

The rates of erythromycin resistance of MRSA were high, ranging from 94.5% to 96.8%, followed by clindamycin resistance (37.4% to 68.9%), fosfomycin resistance (7.7% to 17%), vancomycin

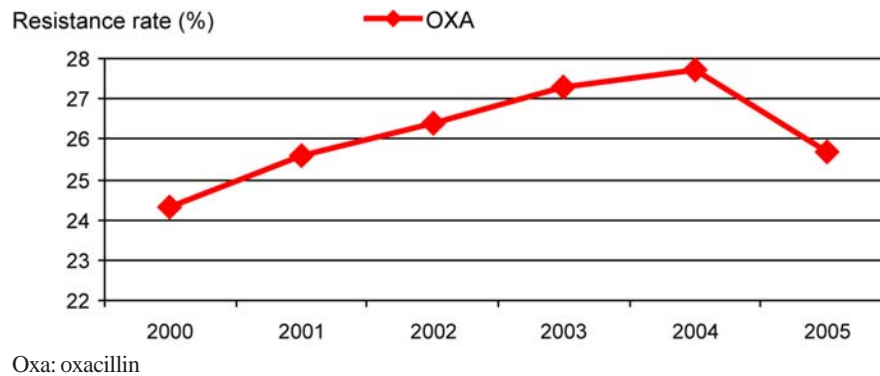


Fig. 1 Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from 2000 to 2005 in Thailand

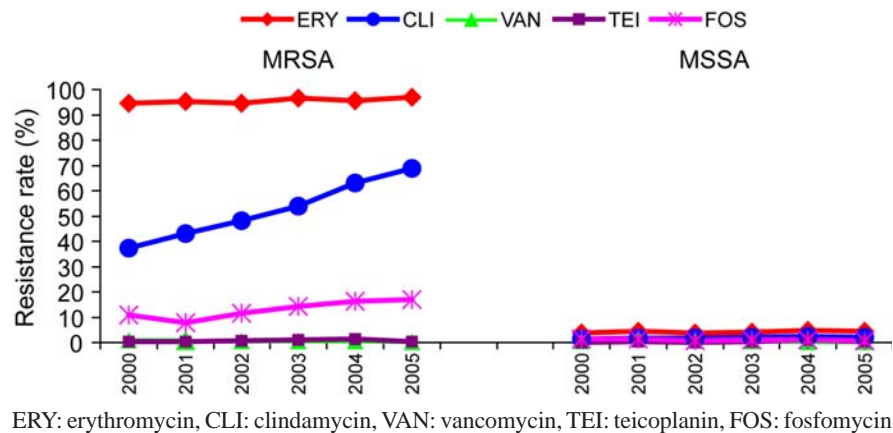


Fig. 2 Rates of antimicrobial resistance of *Staphylococcus aureus* isolated from 2000 to 2005 in Thailand

resistance (0.1% to 0.8%), and teicoplanin resistance (0.2% to 1.3%).

In contrast, the rates of antimicrobial resistance of MSSA were low, with erythromycin resistance ranging from 3.7% to 4.6%, clindamycin resistance ranging from 1.4% to 2.3%, fosfomycin resistance ranging from 0.7% to 1.4%, vancomycin resistance ranging from 0.1% to 1.2%, and teicoplanin resistance ranging from 0.1% to 1.1%.

Rates of antimicrobial resistance of MRSA and MSSA isolates from different parts of Thailand (Fig. 3-4)

The country was divided into five regions including the North, the Northeast, the Center, the East, and the South.

The rates of erythromycin resistance of MRSA ranged from 90.9% to 98.2% among isolates from each region. The rates of clindamycin resistance

ranged from 37.1% to 59.3%, 29.4% to 73.5%, 42.9% to 71.9%, 28.2% to 58.4%, and 45.4% to 76.6% among isolates from the North, the Northeast, the Central, the East, and the South, respectively. The rates of vancomycin resistance ranged from 0.5% to 3.3%, 0% to 0.4%, 0% to 0.9%, 0% to 0.8%, and 0.3% to 0.8%, among isolates from the North, the Northeast, the Central, the East, and the South, respectively. The rates of teicoplanin resistance ranged from 0% to 3.4%, 0% to 0.6%, 0% to 6.9%, and 0% to 2.3%, among isolates from the North, the Central, the East, and the South, respectively. The rates of fosfomycin resistance ranged from 2.9% to 9.7%, 0% to 11.5%, 5% to 46.7%, 7.6% to 23.2%, and 4.8% to 44% among isolates from the North, the Northeast, the Center, the East, and the South, respectively.

The rates of drug resistance of MSSA were less than 10% among isolates from the North, the

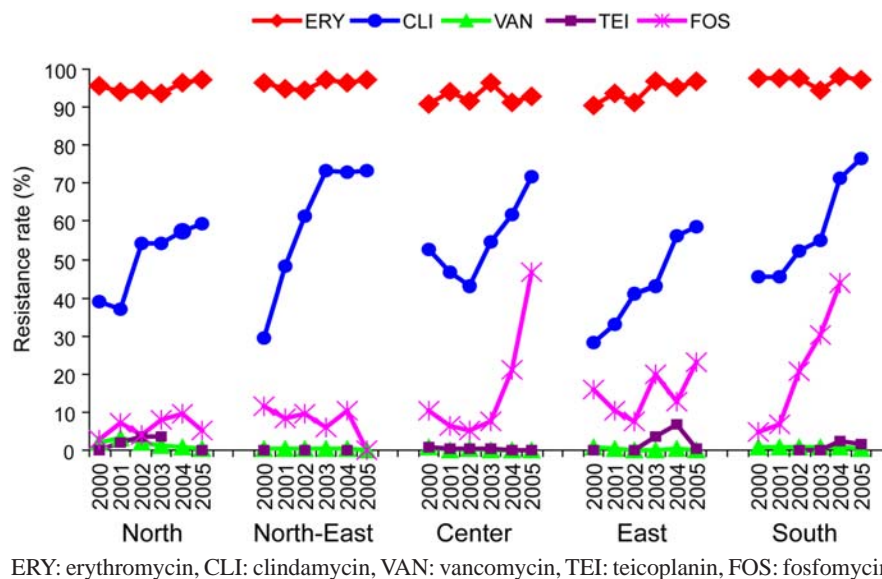


Fig. 3 Rates of antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from different parts of Thailand

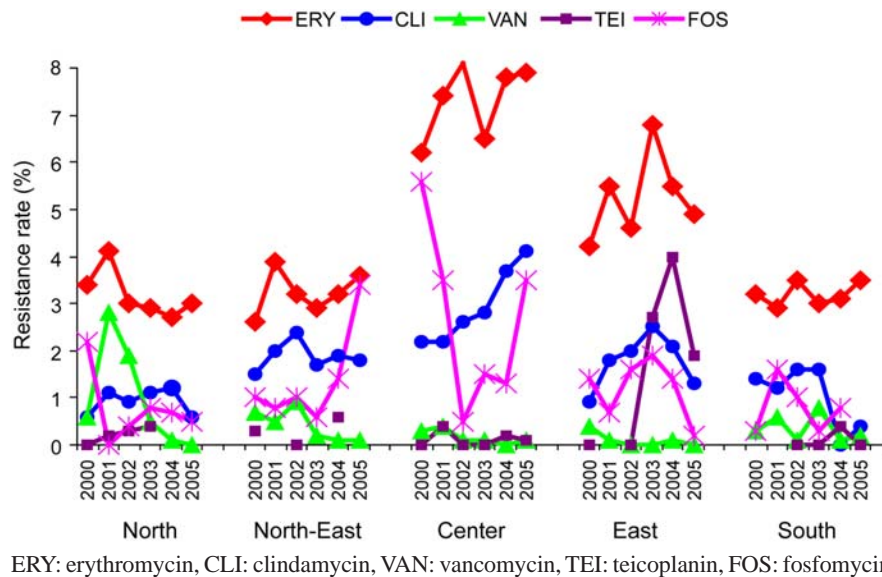


Fig. 4 Rates of antimicrobial resistance of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from different parts of Thailand

Northeast, the Central, the East, and the South, with erythromycin resistance ranging from 2.6% to 8.1%, clindamycin resistance ranging from 0% to 4.1%, fosfomycin resistance ranging from 0% to 5.6%, vancomycin resistance ranging from 0% to 2.8%, and teicoplanin resistance ranging from 0% to 4%.

Rates of antimicrobial resistance of MRSA and MSSA isolates from different types of hospitals in Thailand (Fig. 5-6)

In the present study, there were four types of hospitals including provincial hospital (between 100 and 400 beds), regional hospital (between 400

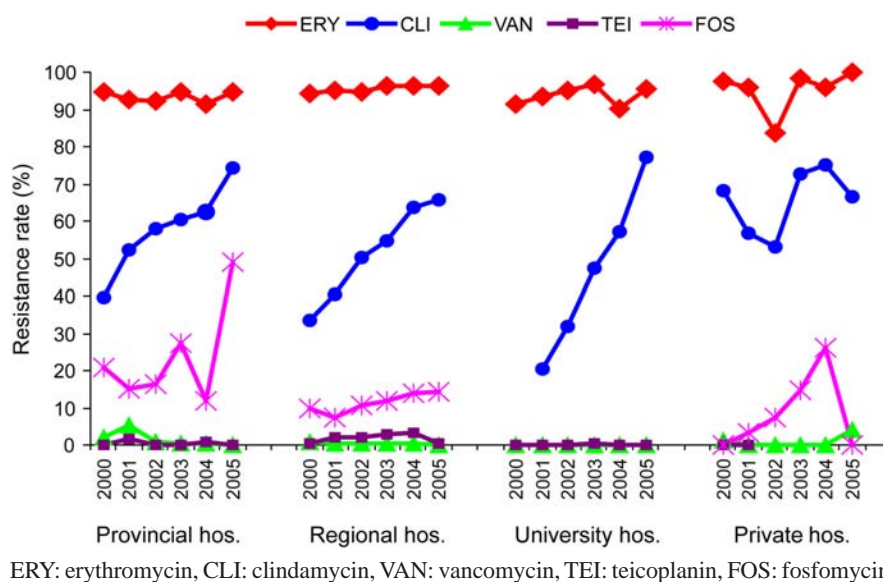


Fig. 5 Rates of antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from different types of hospitals in Thailand

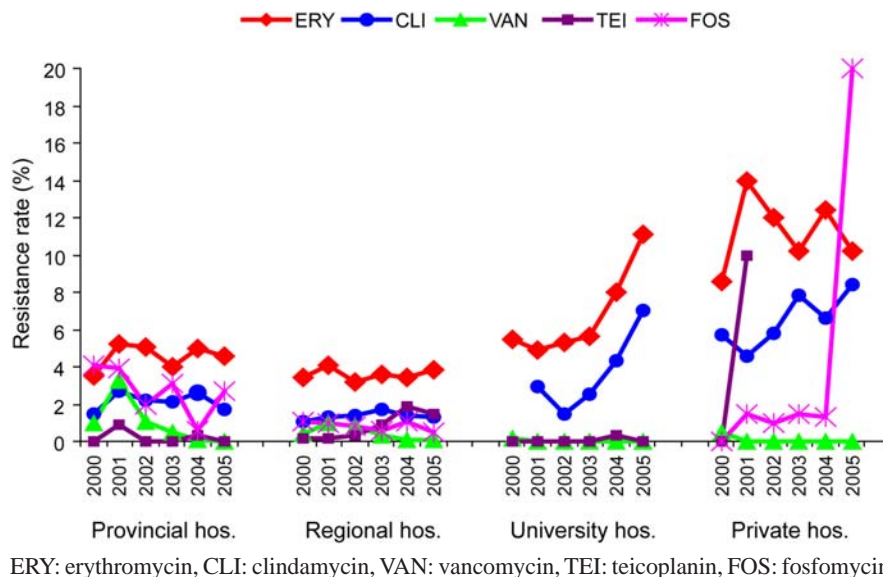


Fig. 6 Rates of antimicrobial resistance of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from different types of hospitals in Thailand

and 1,000 beds), university hospital, and private hospital.

The rates of erythromycin resistance of MRSA ranged from 83.5% to 100% among isolates from each type of hospitals. The rates of clindamycin resistance ranged from 39.5% to 74.2%, 33.6% to

65.8%, 20.3% to 77.2%, and 53.0% to 75.2% among isolates from provincial hospitals, regional hospitals, university hospitals, and private hospitals, respectively. The rates of fosfomycin resistance ranged from 12% to 48.8%, 7.4% to 100%, and 0% to 26% among isolates from provincial hospitals, regional hospitals,

and private hospitals, respectively. The rates of vancomycin resistance ranged from 0% to 5.5%, 0.1% to 0.9%, and 0% to 4% among isolates from provincial hospitals, regional hospitals, and private hospitals, respectively. The rates of teicoplanin resistance were 1.5% and 0.8% in 2001 and 2004, respectively among isolates from provincial hospitals. These rates ranged from 0.6% to 3.2% among isolates from regional hospitals, and only 0.5% rate was noted in 2003 among isolates from university hospitals. There was no teicoplanin resistance among isolates in 2000 and 2001 from private hospitals.

In contrast, the rates of antibiotic resistance in MSSA were low among isolates from provincial hospitals, regional hospitals, university hospitals, and private hospitals. The rates of erythromycin resistance ranged from 3.2% to 14%, followed by clindamycin resistance (1.1% to 8.4%). The rates of fosfomycin resistance ranged from 0% to 20% among isolates from provincial hospitals, regional hospitals, and private hospitals. The rates of vancomycin resistance ranged from 0% to 3.3% and 0.1% to 1% among isolates from provincial hospitals and regional hospitals, respectively. In 2000, the rates of vancomycin resistance were 0.2% and 0.5% among isolates from university hospitals and private hospitals, respectively. In the same year, the rates of teicoplanin resistance ranged from 0% to 0.9% and 0.2% to 1.9% among isolates from provincial

hospitals and regional hospitals, respectively. In 2001 and 2004, the resistance rates of teicoplanin were 10% and 0.3% in 2001 and 2004 among isolates from university hospitals and private hospitals, respectively.

Rates of antimicrobial resistance of MRSA and MSSA isolated from different wards (Fig. 7)

In the present study, clinical isolates in each year were collected from patients in different patient-care areas including out-patient departments (OPDs), in-patient departments (IPDs), and intensive care units (ICUs).

The rates of erythromycin resistance of MRSA were relatively high ranging from 83.3% to 100%, followed by clindamycin resistance ranging from 15% to 69.4%, fosfomycin resistance, vancomycin, and teicoplanin resistance ranging from 0% to 2%, 0% to 3.5%, and 3.4% to 38.7%, respectively, among isolates from OPDs, IPDs, and ICUs.

In contrast, the rates of antimicrobial resistance of MSSA were low, with erythromycin resistance ranging from 1.5% to 5%, and clindamycin resistance ranging from 0% to 2%. The rates of fosfomycin resistance ranged from 0.6% to 2.2%; vancomycin resistance ranged from 0% to 2.5%; and teicoplanin resistance ranged from 0% to 2.6% among isolates from OPDs and IPDs, and no fosfomycin resistance was observed among isolates from ICUs.

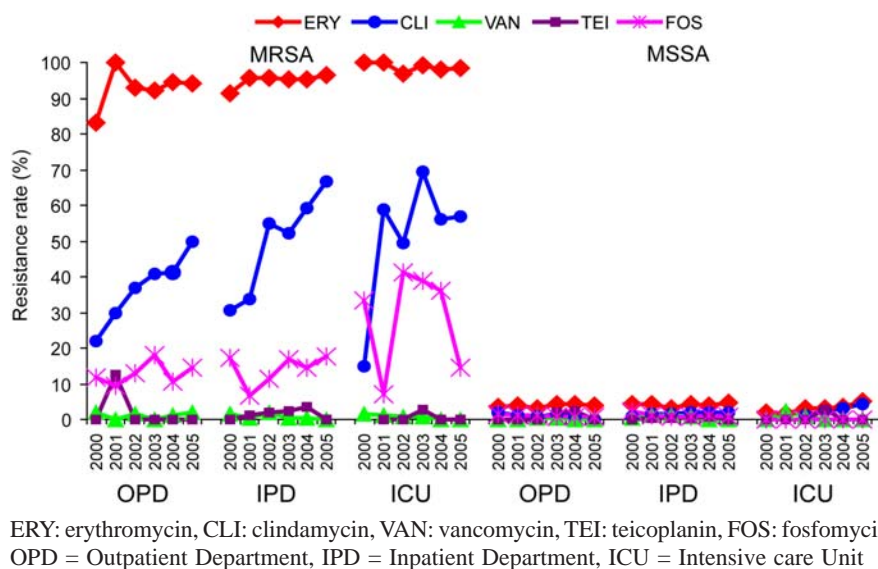


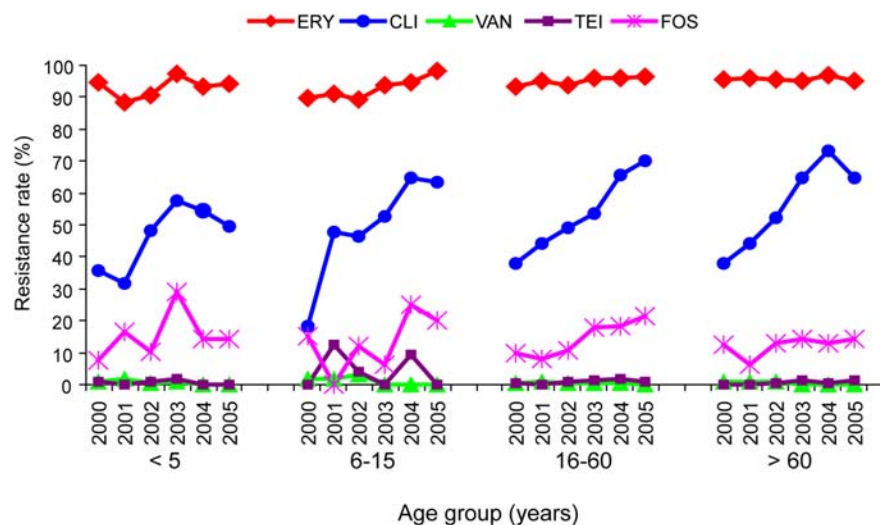
Fig. 7 Rates of antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* isolated from different wards

Rates of antimicrobial resistance of MRSA and MSSA isolated from patients in different age groups in Thailand (Fig. 8-9)

In the present study, the patients were categorized into four age groups including the age

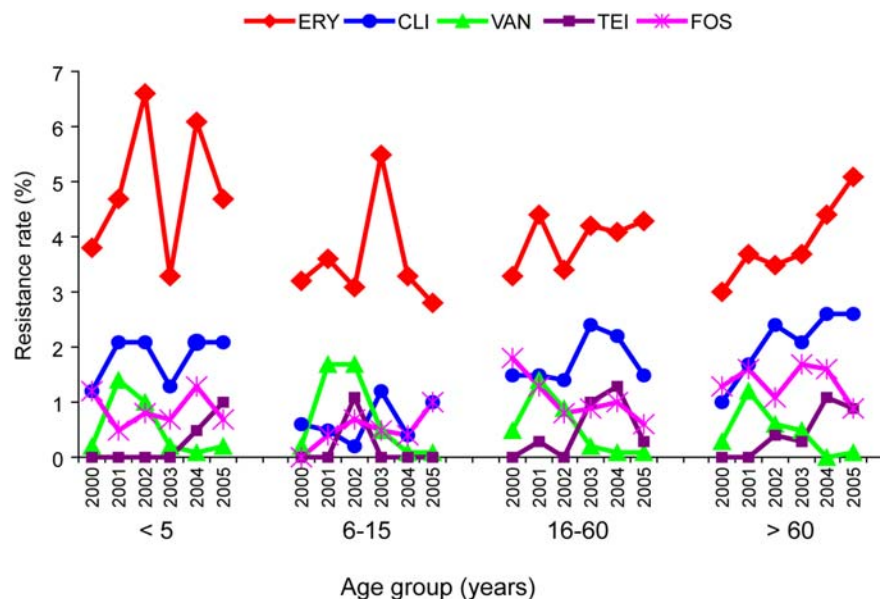
group of younger than five years, between 6-15 years, between 16-60 years, and older than 60 years.

The rates of erythromycin resistance of MRSA were highest, ranging from 88.3% to 98.2%, followed by clindamycin resistance (18.2% to 73%),



ERY: erythromycin, CLI: clindamycin, VAN: vancomycin, TEI: teicoplanin, FOS: fosfomycin

Fig. 8 Rates of antimicrobial resistance of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients of different age groups in Thailand



ERY: erythromycin, CLI: clindamycin, VAN: vancomycin, TEI: teicoplanin, FOS: fosfomycin

Fig. 9 Rates of antimicrobial resistance of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from patients of different age groups in Thailand

fosfomycin resistance (0% to 28.8%), vancomycin resistance (0% to 3%), and teicoplanin resistance (0% to 12.5%) among isolates from the age group of younger than five years, between 6-15 years, between 16-60 years, and older than 60 years.

The rates of erythromycin resistance of MRSA were highest, ranging from 2.8% to 5.9%, followed by clindamycin resistance (0.2% to 2.6%), fosfomycin resistance (0% to 1.8%), vancomycin resistance (0% to 1.6%), and teicoplanin resistance

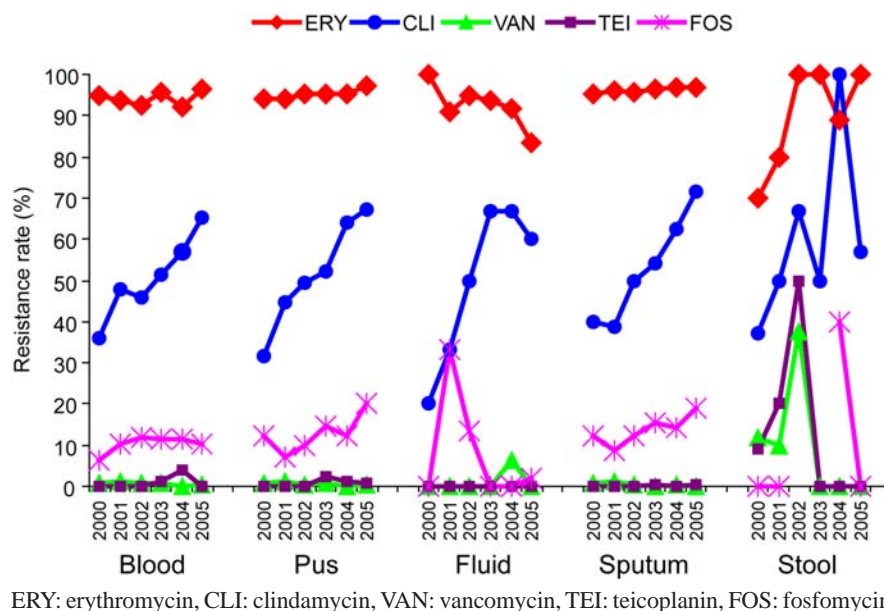


Fig. 10 Rates of antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from different specimens

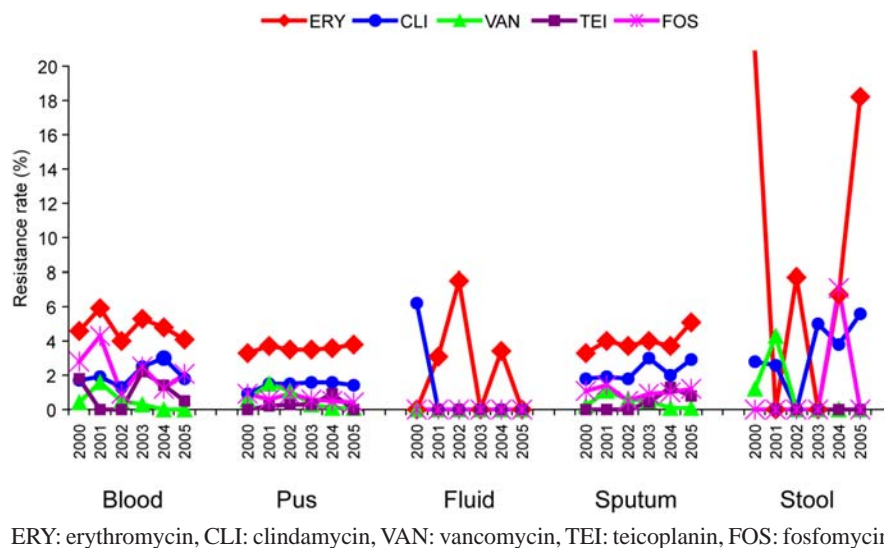


Fig. 11 Rates of antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MSSA) isolated from different specimens

(0% to 1.3%) among isolates from the age group of younger than five years, between 6-15 years, between 16-60 years, and older than 60 years.

Rates of antimicrobial resistance of MRSA and MSSA isolated from patients in different specimens (Fig. 10-11)

The isolates were collected from clinical specimens including the blood, pus (including pus from a wound), body fluids (including amniotic fluid, cerebrospinal fluid, dialysis fluid, gastric fluid, and pleural fluid), sputum, and stool (including rectal swab).

The rates of erythromycin resistance of MRSA were highest ranging from 92.2% to 100%, followed by clindamycin resistance (31.5% to 71.7%), fosfomycin resistance (6.3% to 20%), vancomycin resistance (0% to 37.5%), and teicoplanin resistance (0% to 3.8%) among isolates from the blood, pus, and sputum.

The rates of erythromycin resistance of MSSA were highest ranging from 0% to 5.9%, followed by clindamycin resistance (0.9% to 3.0%), fosfomycin resistance (0.4% to 4.3%), vancomycin resistance (0% to 1.6%), and teicoplanin resistance (0% to 2.2%) among isolates from the blood, pus, and sputum.

For MRSA and MSSA isolates from the body fluids and stool, the comparison could not be made due to the small sample size.

Discussion

In the present study, the data from 28 hospitals including the types of specimen, bacterial isolation, and diameter of antimicrobial inhibition zone were sent to NARST center (Nonthaburi, Thailand), and the analysis was performed by using WHONET software program. This study showed the relatively constant rates of MRSA, in contrast to the study by Thamlikitkul et al which showed the rates of MRSA ranged from 30 to 60 %. This difference is likely due to different *S. aureus* isolates studied, both community and nosocomial strains in the present study and only nosocomial strains in the latter study⁽¹⁰⁾. Carrier rates are between 11% and 32% among healthy adults in the general population and a prevalence rate of 25% was found among hospital personnel⁽¹⁹⁾. Approximately 85% of carriers can be identified with a swab taken from the anterior nares. Higher carrier rates are seen in injection drug users, patients with dermatological conditions and in patients with long term indwelling intravascular catheters. The transmission of MRSA from temporary colonization of the hands of healthcare

workers is the major mechanism of spread of MRSA in hospitals at present.

The NARST data showed that within all regions, all age groups, and all wards, there were similar rates of drug resistance of MRSA and MSSA. The rates of erythromycin resistance of MRSA were constantly high, ranging from 83.3% to 100% during 2000 and 2005 among isolates from all regions, all hospital types and all age groups. The rates of clindamycin resistance of MRSA increased from 37.4% to 68.9% from 2000 to 2005. This left all glycopeptides including vancomycin and teicoplanin as the only effective agents available at the time. The present study showed that vancomycin resistance of MRSA ranged from 0.1% to 0.8%, parallel to those of MSSA which ranged from 0.1% to 1.2%. Interestingly, there was an increase of MRSA isolated from the stool specimens which had vancomycin resistance rate of 11.8% (4 of 34 patients) in 2000, 10% (1 of 10 patients) in 2001, and 37.5% (3 of 8 patients) in 2002. Whereas the rates of vancomycin resistance among MSSA isolated from the stool specimens were 1.2% in 2000 and 4.3% in 2001. Surprisingly, the authors found that some MRSA and MSSA isolates resisted to both vancomycin and teicoplanin, in the range of 0.1% to 1.2%. To our knowledge, such resistance has never been reported in Thailand. As recommended by the CLSI, the identification of VRSA or VISA must be determined by the broth dilution method. Thus, at the moment, the authors have to further confirm our results for the occurrence of VRSA or VISA by the recommended method before making a definite conclusion.

The heterogeneously intermediate resistance to vancomycin was variably reported among MRSA isolates from Japan (8.2%), India (6.3%), South Korea (6.1%), the Philippines (3.6%), Vietnam (2.4%), Singapore (2.3%), and Thailand (2.1%), but it was not observed among strains from China, Indonesia, Saudi Arabia, Sri Lanka, and Taiwan⁽²⁰⁾. Unfortunately, the present study was not planned for detecting vancomycin-resistant *S. aureus* (VRSA) isolates.

In conclusion, the prevalence and antimicrobial susceptibility of MRSA and MSSA isolates from all regions, all hospitals, all age groups, all wards, and all specimens were quite comparable and stable during 2000 and 2005. Around 26% of overall *S. aureus* isolates were MRSA have been noted. In addition, the present study provided no concrete evidence on VRSA, vancomycin-intermediate *S. aureus* (VISA), and heterogeneous VISA. Furthermore, the emergence

and magnified of clinical problems related to community-acquired MRSA needs to be monitored. Moreover, more understanding of epidemiological association between the isolates and molecular typing methods is needed.

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การดื้อยาของเชื้อสแตปฟีโลคอคคัส ออเรียส ที่แยกจากผู้ป่วยในประเทศไทย ระหว่างปี พ.ศ. 2543 ถึง พ.ศ. 2549

ประภาวดี ดิษยาธิคม, สุรางค์ เดชศิริเลิศ, อรทัย ทองมะลิ, ปฐม สวรรค์ปัญญาเลิศ, นลินี อัสวโกศิ, ณะพันธ์ พิบูลย์บรรณกิจ

จากการรวบรวมข้อมูลระหว่างปี พ.ศ. 2543 ถึง พ.ศ. 2549 ของเชื้อสแตปฟีโลคอคคัส ออเรียส ทั้งเชื้อสแตปฟีโลคอคคัส ออเรียส ที่มีความไวต่อยาเมธิซิลิน และเชื้อสแตปฟีโลคอคคัส ออเรียส ที่ดื้อต่อยาเมธิซิลิน ซึ่งแยกได้จากผู้ป่วย จากโรงพยาบาลที่เป็นเครือข่าย ของโครงการเฝ้าระวังเชื้อดื้อยาแห่งชาติในประเทศไทยจำนวน 28 แห่ง ทำการวิเคราะห์ข้อมูลโดยใช้โปรแกรมซอฟต์แวร์ของ WHONET เพื่อดูความชุกและรูปแบบความไวต่อสารต้านจุลชีพของเชื้อ พบเป็นเชื้อสแตปฟีโลคอคคัส ออเรียสดื้อต่อยาเมธิซิลิน ในแต่ละปีโดยเฉลี่ยประมาณร้อยละ 26 ของเชื้อที่แยกได้จากผู้ป่วยทั้งหมด เชื้อสแตปฟีโลคอคคัส ออเรียสดื้อต่อยาเมธิซิลินมีอัตราการดื้อยาอิริโทรมัยซินค่อนข้างสูงอยู่ในช่วงร้อยละ 94.5 ถึง 96.8 ดื้อต่อยา คลินดามัยซินอยู่ในช่วงร้อยละ 37.4 ถึง 68.9 ดื้อต่อแวนโคมัยซินอยู่ในช่วงร้อยละ 0.1 ถึง 0.8 ดื้อต่อยา ไทโครแพลงนินอยู่ในช่วงร้อยละ 0.2 ถึง 1.3 และดื้อต่อยาฟอสโฟมัยซินอยู่ในช่วงร้อยละ 7.7 ถึง 17 ในขณะที่เชื้อสแตปฟีโลคอคคัส ออเรียส ที่มีความไวต่อยาเมธิซิลินมีอัตราการดื้อยาทุกชนิดค่อนข้างต่ำ โดยมีอัตราการดื้อยาอิริโทรมัยซินอยู่ในช่วงร้อยละ 3.7 ถึง 4.6 ดื้อต่อยาคลินดามัยซินอยู่ในช่วงร้อยละ 1.4 ถึง 2.3 ดื้อต่อแวนโคมัยซินอยู่ในช่วงร้อยละ 0.1 ถึง 1.2 ดื้อต่อยาไทโครแพลงนินอยู่ในช่วงร้อยละ 0.1 ถึง 1.1 และดื้อต่อยาฟอสโฟมัยซินอยู่ในช่วงร้อยละ 0.7 ถึง 1.4 จากการพบเชื้อสแตปฟีโลคอคคัส ออเรียสดื้อต่อแวนโคมัยซินเพิ่มขึ้น ชี้ให้เห็นว่าควรมีการตรวจสอบความถูกต้องของผลการทดสอบความไวเชื้อต่อยาแวนโคมัยซินเพื่อยืนยันผลซ้ำอีกครั้ง และการจำแนกชนิดทางพันธุกรรมจะทำให้มีความเข้าใจในความสัมพันธ์ด้านระบาดวิทยาของเชื้อเพิ่มขึ้น
