Case Report

The First Pediatric Case of Staphylococcus aureus With Heterogenous Resistant to Vancomycin Endocarditis in Thailand

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Staphylococcus aureus *with reduced susceptibility to vancomycin has been reports worldwide. Here we report the first pediatric case of heterogeneous vancomycin intermediate resistance* Staphylacoccus aureus *(hVISA) causing endocarditis in Thailand. A 4 months old girl with truncus arteriosus type IV and ventricular septal defect developed methicillin-resistant S. aureus (MRSA) bacteremia and endocarditis after total repair operation. The patient did not respond to combination antimicrobial treatment including vancomycin. The strain was susceptible to trimethoprim-sulfamethoxazole and vancomycin by conventional antimicrobial susceptibily test. The vancomycin minimal inhibitory concentration by E-test was 2 μg/ml. The strain was judged to be possible heteroresistant when screening was done by one-point population analysis. The subsequent population analysis and testing for the emergence of mutants with reduced susceptible to vancomycin confirmed that this strain was hVISA. Despite the treatment with vancomycin, amikacin, rifampicin and cotrimoxazole, the patient died.*

hVISA should be suspected in MRSA infections that were refractory to vancomycin therapy could be due to. The emergence hVISA underscored the importance of the prudent use of antibiotics, the laboratory capacity to identify MRSA and hVISA and proper communication with treating clinicians, and the meticulous infection-control measures to prevent transmission.

Keywords: *hVISA, VISA, GISA, MRSA, Staphylococcus aureus, Endocarditis*

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Staphylococcus aureus is an important cause of serious infections in children, including pneumonia, bone and joint infections, soft tissue infections and endocarditis. In the 1980s, methicillin-resistant *S. aureus* (MRSA) emerged; leading to increasing use of vancomycin[1]. In 1997, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin (MIC 8 μg/ml; strain Mμ 50) was reported from Japan[2]. Subsequently, vancomycin or glycopeptide intermediately resistant *S. aureus* (VISA/GISA) and heterogeneous resistance to vancomycin (hVISA) have been identified in the United States[3-5], Asia[6-8], and Europe[9-13]. To date, three documented cases of infection caused by vancomycin-resistant *S. aureus* (VRSA) have been reported in the United States[14-16]. In Thailand, there was a report of MRSA with reduced susceptibility to vancomycin in 3 adult patients in 2001[17]. Here we report the first pediatric case of infection with hVISA endocarditis in Thailand.

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Case Report

In March 2002, a 4 month-old girl with truncus arteriosus type IV and ventricular septal defect devel-

oped MRSA bacteremia and endocarditis 20 days after total repair operation with homograft. MRSA was isolated from her blood after prolonged exposure to various antibiotics, i.e, cefotaxime, cefipime, meropenem and 7 day-course of vancomycin. Three subsequent blood specimens grew MRSA and she had persistent fever even after 10 days of the combination treatment with vancomycin, amikacin and rifampicin. The vancomycin minimal inhibitory concentration (MIC) was 2 μg/ml by E-test. This MRSA strain was susceptible to cotrimoxazole; therefore, cotrimoxazole was added to the treatment regimen. Bacteremia was cured and fever subsided 10 days thereafter, but three repeated echocar-diogram demonstrated enlarging vegetation at homo-graft. Despite the treatment with vancomycin, amikacin, rifampicin and cotrimoxazole, the patient died 48 days thereafter.

Materials and Methods

Definition of hVISA

In the United States, The National Committee for Clinical Laboratory Standards (NCCLS) has developed guidelines to define susceptibility for *S. aureus.* Isolates for which MIC of vancomycin ≤ 4μg/ml are susceptible, and isolates for which the MIC of vancomycin is 8-16 μg/ml are intermediate. Resistance is defined as an MIC of vancomycin of ≥ 32 μg/ml[18].

hVISA strains are defined as strains of *S. aureus* that contain subpopulations of vancomycin-intermediate daughter cells but for which the MICs of vancomycin for the parent strain are only 1-4 μg/ml. The prototype strains (Mμ3) was described by Hiramatsu et al[6].

Bacterial strain

P2 was an MRSA strain isolated from blood of this patient. ATCC 29213 was a representative vancomycin susceptible *S. aureus* (VSSA) strain, and Mμ3 (ATCC 700698) was the strain isolated in Japan in 1996 with heterogeneous resistance to vancomycin. Mμ50 (ATCC 700699), isolated in Japan in 1996, was the first VRSA. Both of Mμ3 and Mμ50 were used as positive control strains of heterogenous and homogeneous vancomycin resistance, respectively.

Screening of hVISA

Screening was done by one-point population analysis method proposed by Hiramatsu et al[6]. The strain was judged to be possible heteroresistant if a countable number (1-30) of colonies was apparent within 48 hours: hVISA was considered definite if the strain produced a subclone(s) with vancomycin MIC of 8 μg/ml or above upon selection with vancomycin, and based on population curve with a heterogeneous pattern, and a positive result in resistant mutant emergence test.

Resistant-mutant emergence test

Subclones of P2 was selected from brain heart infusion (BHI) agar plate containing 4 μg/ml of vancomycin and subculture on the drug free medium. The mutant strain was cultivated overinght in BHI broth. The MIC of vancomycin was determined using the agar dilution method with 1 μg/ml increments of vancomycin in the range 1-9 μg/ml.

Population analysis

100 μl of a starting cell suspension, corresponding to an optical density at 578 nm of 0.3, and 7-fold serial dilution were spread on BHI agar plates containing various concentrations of vancomycin. After incubation for 48 hours, the colonies on each plate were counted. The number of resistant cells contained in 100 μl of starting cell suspension was calculated and plotted on a semilogarithmic graph.

Results

Vancomycin susceptibility of P2 strain

Base on the NCCLS criteria, P2 strain were judged to be susceptible to vancomycin by E test (vantomycin MIC = 2 μg/ml) and disk-diffusion susceptibility tests.

Analysis of vancomycin-resistant subpopulations of the P2 MRSA strain

With an inoculum size of 10^6 cfu/ml, 8 colonies of P2 grew on BHI agar containing 4 μg/ml of vancomycin within 48 hours, reflecting the presence of vancomycin resistant subpopulation.

Mutants with vancomycin MIC of 8 μg/ml was obtained from P2 strain by this one-step selection procedure.

Discussion

Although the incidence of VISA and VRSA remains low, there has been an increasing number of reports of hVISA from several countries. Song JH and the Asian Network for Surveillance of Resistant Pathogens (ANSORP) study group reported 4.3% prevalence of hVISA among 1,357 clinical isolates of MRSA collected from 12 Asian countries between January 1997 and March 2000. Of 96 MRSA isolates from Thailand, 2

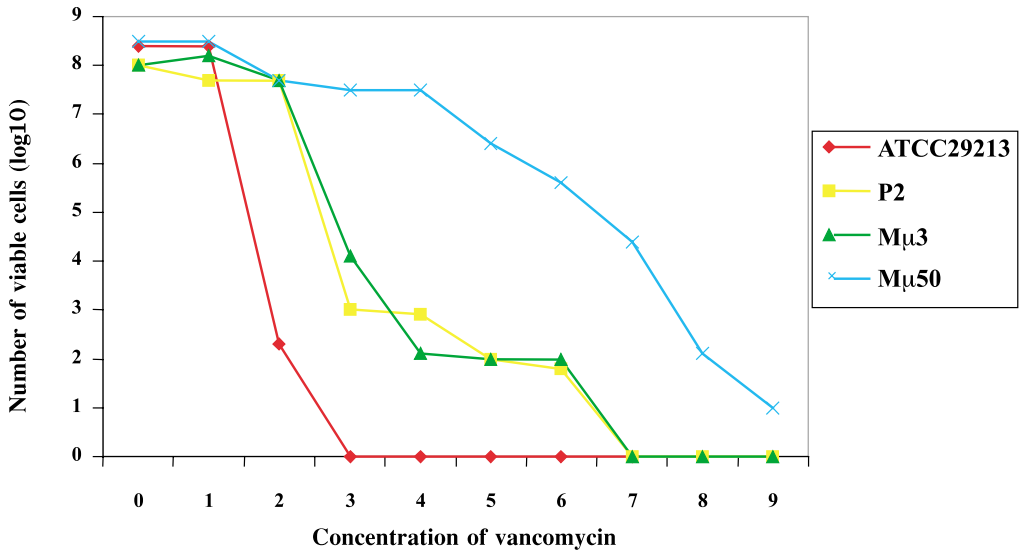

Fig. 1 Comparison of the population analysis of Mμ3, Mμ50, P2 and VSSA strain, ATCC29213. Mμ3 and P2 contained resistant subpopulation that grew in the present of 5-7 μg/ml of vancomycin

(2.1%) were hVISA. Fortunately, neither VISA nor VRSA isolates were found among isolates from Asian countries among this survey[8]. However, the emergence of VISA or VRSA strains in this region can be anticipated.

The proposed mechanism of reduced susceptibility to vancomycin of *S. aureus* is the thickening of cell wall. In addition, the increased production of nonamidated muropeptides may also contribute to vancomycin resistance by increasing the efficiency of affinity trapping and clogging of the mesh of the peptidoglycan outer layers. The thicker the cell wall, the vancomycin molecules would be trapped within the cell wall, thus allowing a decreased number of vancomycin molecules to reach the cytoplasmic membrane where the real functional targets of vancomycin are present[19,20]. Cui, et al have demonstrated that cell wall thickness had high correlation with the MICs of the two glycopeptides (correlation coefficient = 0.908 for vancomycin and = 0.655 for teicoplanin)[20].

Fridkin, et al reported that the risk factors for infection with *S. aureus* with reduced susceptibility to vancomycin[21] were the antecedent use of vancomycin in prior month (OR = 13; 95% CI 1.8-100) and in 3-6 months (OR = 2.8; 95% CI 1.1-7.0), and the isolation of MRSA from a culture in prior 2-3 months (OR = 32.5; 95% CI 1.1-947). The prior use of vancomycin may be the risk factor in this patient.

The clinical significance of hVISA strains is still controversial. However, there have been reports of vancomycin treatment failure, some with fatal outcome associated with hVISA[17, 22-24]. Wong, et al reported that the overall mortality from bacteremia due to *S. aureus* with reduced susceptibility to vancomycin was higher than that in patient with vancomycin-sensitive isolates (44% vs 10%)[25].

Unfortunately, conventional antimicrobial susceptibility test cannot detect hVISA. Clinicians should keep in mind that MRSA infections that were refractory to vancomycin therapy could be due to hVISA[26].

Currently, there is no guidelines for treatment of infection due to *S. aureus* with reduced susceptibility to vancomycin. Removal of infected indwelling hardware and debridement of infected sites is most importance and must be considered in every patient[27]. Most isolates of hVISA (87%) have been susceptible to trimethoprim-sulfamethoxazole (TMP-SMX)[28], and this agent has been used in various combinations for the treatment of *S. aureus* with reduced susceptibility to vancomycin. The addition of rifampicin to therapy can also be considered if the isolate is susceptible[27]. We were able to eradicate bacteremia in this patient with this combination antibiotics, however, the vegetation persisted, and resulted in a fatal outcome. Several new drugs such as linezolid, quinopristin-dalfopristin and daptomycin have shown promising in the treatment of infection with *S. aureus* with reduced susceptibility to vancomycin. In addition, decolonization should be attempted with mupirocin if the patient is colonized with

this organism in the anterior nares and chlorhexidine washes can be considered in select cases[27].

A patient infected with h VISA and VRSA should be placed in isolated room and have dedicated patient-care items. Health-care workers providing care to such patients should follow contact precautions[29].

Conclusion

The emergence of *S. aureus* with reduced susceptibility to vancomycin underscored the importance of the prudent use of antibiotics, the laboratory capacity to identify resistant strain and the meticulous infection-control measures to prevent transmission.

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รายงานผู้ป่วยเด็กที่มีภาวะการติดเชื้อของเยื่อบุหัวใจจากเชื้อสแตฟฟิโลคอคคัสออเรียสซึ่งดื้อต่อยาแวนโคมัยซินรายแรกในประเทศไทย

วนัทปรียา พงษ์สามารถ, สมพร ศรีเฟื่องฟุ้ง, จันทิมา เธียรศศิธร, สเน่ห์ เจียสกุล, นิรันดร์ วรรณประภา, กุลกัญญา โชคไพบูลย์กิจ.

มีรายงานการติดเชื้อ สแตฟฟิโลคอคคัส ออเรียส ซึ่งมีความไวต่อยาแวนโคมัยซินลดลงในหลายประเทศทั่วโลก รายงานนี้เป็นผู้ป่วยเด็กที่มีภาวะการติดเชื้อของเยื่อบุหัวใจจากเชื้อสแตฟฟิโลคอคคัสออเรียสซึ่งดื้อต่อยาแวนโคมัยซินรายแรกในประเทศไทย เด็กหญิงไทยอายุ 4 เดือนซึ่งมีความพิการของหัวใจแต่กำเนิดมีปัญหาติดเชื้อในกระแสเลือดและเยื่อบุหัวใจจากเชื้อสแตฟฟิโลคอคคัสออเรียสซึ่งดื้อต่อยาเมธิซิลลิน ผู้ป่วยไม่ตอบสนองต่อการรักษาด้วยยาปฏิชีวนะหลายขนานซึ่งมียาแวนโคมัยซินรวมอยู่ด้วย เชื้อนี้ไวต่อยาตรัยเมโทรพริม-ซัลฟาเมธอกซาโซลและแวนโคมัยซินโดยการทดสอบความไวต่อยาปฏิชีวนะด้วยวิธีมาตรฐาน โดยมีค่าความเข้มข้นของยาแวนโคมัยซินน้อยสุดที่สามารถยับยั้งการเจริญเติบโตของเชื้อที่ 2 ไมโครกรัมต่อมิลลิลิตรโดยวิธีอีเทส เมื่อทำการคัดกรองพบว่าเชื้ออาจดื้อต่อยาแวนโคมัยซิน เมื่อทำการทดสอบเพิ่มเติมสามารถยืนยันได้ว่าเชื้อสายพันธุ์นี้ดื้อต่อแวนโคมัยซิน แบบไม่สม่ำเสมอ ผู้ป่วยรายนี้เสียชีวิตแม้จะได้รับการรักษาด้วยยาแวนโคมัยซินร่วมกับอมิเคซิน ไรแฟมพิซิล และโคตรัยมอกซาโซล ควรคิดถึงถึงเชื้อสแตฟฟิโลคอคคัสออเรียสซึ่งมีความไวต่อยาแวนโคมัยซินลดลง หากผู้ป่วยไม่ตอบสนองต่อการรักษาด้วยยาแวนโคมัยซิน และตระหนักถึงการใช้ยาปฏิชีวนะอย่างเหมาะสม มีความจำเป็นที่ห้องปฏิบัติการควรมีศักยภาพในการตรวจหาเชื้อดื้อยาเหล่านี้ และสื่อสารให้แพทย์ทราบ และต้องเคร่งครัดในมาตรการเพื่อป้องกันและควบคุมการแพร่ระบาดของการติดเชื้อในโรงพยาบาล