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Vancomycin intermediate-Resistant Staphylococcus aureus (VISA) Isolated from a patient who never received Vancomycin treatment

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SUMMARY

Background: With the abuse of antibiotics, the methicillin-resistant *Staphylococcus aureus* (MRSA) strain became prevalent. Furthermore, *Staphylococcus aureus* with a character of vancomycin intermediate-resistance (VISA) has been found globally since the first report in Japan. The main objectives of this study were to report a case of VISA isolated from a Chinese patient who had never undergone Vancomycin treatment, and to determine its molecular character.

Methods: A total of 9 strains were recovered from a patient during the therapeutic process. Antimicrobial susceptibility testing was performed to determine their antibiotic susceptibility patterns. To detect the VISA strain's molecular epidemiological features, growth and morphological characters, we used multilocus sequence typing, autolysis assay and transmission electric microscope tests. Pulsed-field gel electrophoresis (PFGE) was performed to characterize the heterogeneities of all isolates.

Results: One isolate was found to exhibit vancomycin intermediated-resistant with MIC of 8 μg/ml. It was ST239-T030-agr-1, had thickened cell wall, and displayed a slower growth rate and reduced susceptibility to Triton X-100-induced autolysis than other strains. All 9 strains exhibited the same PFGE pattern.

Conclusion: This is the first report of VISA found in central China from a patient who had never received vancomycin treatment.

1. Introduction

Staphylococcus aureus remains a dominant cause of bacteraemia worldwide due to its ability to adapt to different environments, even to cope with antibiotic pressure.[1] With the abuse of antibiotics, the methicillin-resistant *Staphylococcus aureus* (MRSA) strain became more prevalent. The prevalence of MRSA even exceeded 70% of all *S.aureus* in Asia.[2–4]

MRSA infection is resistant to methicillin and related β-lactam antibiotics. The therapy of MRSA infections began to shift to the use of glycopeptide antibiotics in the 1980s, particularly Vancomycin. Unfortunately, under the pressure of glycopeptide antibiotics, Vancomycin intermediate-resistant *Staphylococcus aureus* (VISA) emerged and was firstly reported in Japan in 1997.[5] Since then VISA has been isolated with increasing frequency from several hospitals around the world.[6–8] Mainland China also faced the same problem, as indicated by Sun W's work.[9] In most cases of nosocomial VISA infection, Vancomycin application could be found. In the present study, we report a first VISA isolated from a patient who received teicoplanin, Linezolid and other antibiotics, but was never treated with Vancomycin.

As part of the study, we subjected all *S.aureus* strains from this patient to antibiotic susceptibility testing, molecular typing, and PFGE analysis. The VISA strain was further characterized by the cell-wall thickening test and autolysis detection.

2. Materials and methods

2.1. Bacterial strains and patient

The VISA isolate was recovered from a large teaching hospital with 3700 beds located in Central-Southern China (Tongji hospital, Wuhan, China). The patient, a 51-year-old man, was admitted to our hospital on Mar 18th, 2013 with a complaint of dysphagia for one month, but no history of hospitalization. A presumptive

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diagnosis of Esophageal cancer was made by gastroscopy examination. Then the patient underwent total gastrectomy, subtotal esophageal resection via left chest and esophageal ostomy in the left neck on Mar 21st. Cefoperazone/Tazobactam was administered for 6 days as prophylaxis. However, MRSA isolates, number 5135 (Mar 25th) and number 5247 (Mar 28th), were recovered from the sputum specimens. On Mar 27th, the patient started with Teicoplanin and Piperacillin/Tazobactam for 8 days. The patient's condition was improving. On Apr 4th, the patient underwent the second surgery, colon interposition reconstruction for oesophageal replacement via the left neck and digestive tract reconstruction. Cefoperazone/Tazobactam and Teicoplanin were administrated for 14 days. However, on Apr 6th and Apr 17th, MRSA 5493 and heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA) 5916 were recovered from sputum specimens, respectively. On Apr 17th, stoma fistula was found with high WBC of 11.3×10^9/L and neutrophils of 82.0%. On the second day, VISA isolate 5944 was recovered from drainage of the stoma fistula. Then the antibiotics were changed to Meropenem and Linezolid for 9 days. At the same time, the patient was isolated in a private room and a dedicated nurse was assigned to take care of him. In this stage, a continuous drainage tube with negative pressure was set to control the infection. Eight days later, the amount of drainage decreased and the inflammation was relieved. The stoma fistula was constrained and enwrapped. The neck wound healed well. Decreased WBC of 8.6×10^9/L and N% of 74.0% were achieved. On Apr 27th, the regimen was changed back to Cefoperazone/Tazobactam and Teicoplanin for the other days. On May 3rd, the hospital infection control staff collected four specimens from this patient, including nasal swabs (5964), secretions from the neck wound (5966), anal swabs(5967) and sputum(5968). hVISA were recovered from all these specimens. The patient abandoned treatment and checked out on May 4th for economic reasons. The summary of the patient's course is presented in Figure 1. The control strain Mu3 and Mu50 used in this study were a kind gift from Professor Jingyun Li at the National Institutes for Food and Drug Control.

Although the patient was not treated with Vancomycin during the whole process in the present study, the use of Vancomycin in this hospital is about 4.49 DDDs/100 admissions and the prevalence of MRSA isolates was 69.5% in 2013. The percentage of hVISA in MRSA isolates was considerably high in Central-Southern China, up to 22.1%.[10]

2.2. Antimicrobial susceptibility testing

Identification of *S. aureus* isolates was performed using standard methods and the Vitek 2 compact automated system (bioMérieux, Marcy-l'Étoile, France). Minimum inhibitory concentrations (MICs) of antibiotics were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute recommendations. Reference strain ATCC 29213 was used as control. E-test analysis of vancomycin and teicoplanin were performed using E-test strips (bioMeriux, Durham, NC) according to the manufacturer's instructions.

2.3. Modified population analysis profiling/area under the curve (PAP/AUC)

PAP/AUC was performed as previously described by Wootton et al.[11] Briefly, following 24 h incubation in Tryptone Soya Broth (Oxoid, Basingstoke, England), neat culture and dilutions of 10^{-3} (10^5 CFU/ml) and10^{-6} (10^2 CFU/ml) were plated onto Brain Heart Infusion agar (Oxoid) plates containing 0.0, 0.5, 1.0, 2.0, 2.5, 4.0, and 8.0 mg/L vancomycin. Colonies were counted after 48 h. Control strains (Mu50, and Mu3) were included with each run. The calculated CFU/mL values were plotted against vancomycin concentration using Prism version 5.0 (GraphPad Software, San Diego, CA, USA). The ratio of the AUC of the test isolate to the AUC of Mu3 was calculated and interpreted as follows: for VSSA, a ratio of <0.9; for hVISA, a ratio of 0.9 to 1.3; and for VISA, a ratio of ≥ 1.3.

2.4. DNA isolation

All isolates were cultured on blood agar and incubated overnight at 37 °C. Genomic DNA was extracted using Puregene Yeast/Bact. Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol for Gram-positive bacteria. DNA samples were stored at -20 °C until testing.

2.5. Molecular typing

Multiplex PCR was used to amplify the mecA gene and determine the *staphylococcal* chromosome cassette mec (*SCCmec*) type (I-V) of all MRSA isolates, according to the method published by Milheirico *et al.*[12] Multilocus sequence typing (MLST) was

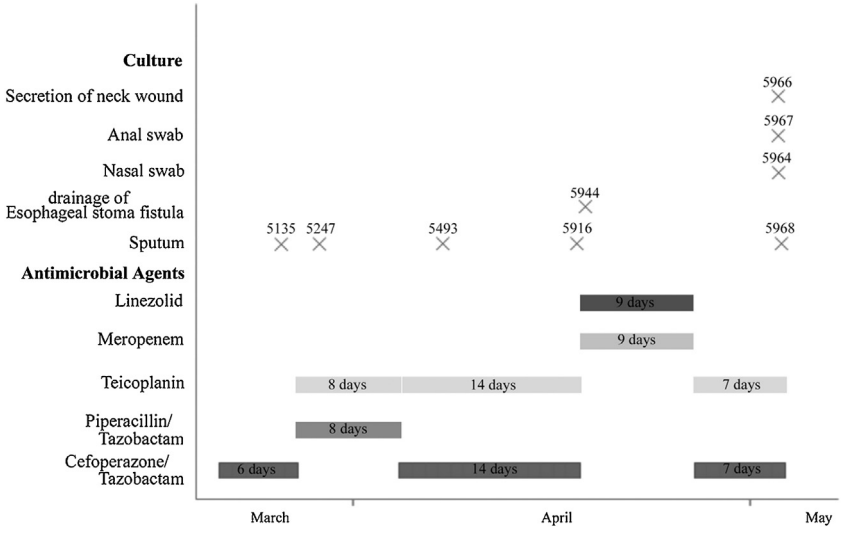

Figure 1. The patient's clinical course. (The lower panel) The antibiotics used are depicted in rectangles. The number of days in each rectangle corresponds to the time of treatment with the antibiotics. (The top panel), All isolates are shown in the order in which they were recovered.

examined as previously described.[13] PCR fragments of 7 housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, and yqiL) were obtained from chromosomal DNA and were directly sequenced. MLST allele names and sequence types were derived from the MLST database (http://www.mlst.net/dbqry/saureus/htm). The accessory gene regulator (*agr*) polymorphism was determined by multiplex PCR using a previously described method.[14] Spa typing was performed as described by Harmsen et al,[15] the spa types with identical or similar repeat profiles were grouped into clusters (http://www.ridom.de/spaserver).

2.6. Detection of the van genes

The vancomycin-resistant genes, including VanA, VanB, VanC1, VanC2 and VanC3, were detected using a multiplex PCR as previously described.[16] The PCR cycle was as follows: 1 cycle of 5 min at 94 °C, then 35 cycles of 94 °C, 1 min, 58 °C, 1 min, and 72 °C 1 min; followed by a final 10 min extension at 72 °C.

2.7. Autolysis assay

Triton X-100-stimulated autolysis in glycin buffer (pH8.0) was measured as described previously.[17] Cells were grown exponentially to an OD_{600} of about 0.3. The cultures were then rapidly chilled, and cells were washed twice with ice-cold distilled water and resuspended to an OD_{600} of 1.2 in 50 mM glycine–0.01% Tris X-100 buffer. Autolysis was measured every 30 min for 4 h in absorbance at OD_{600}. The percentage of the remaining optical density at each time point was plotted.

2.8. Electron microscopic evaluation of cell wall thickness

Electron microscopy was performed as described previously by Cui et al.[18] Briefly, mid-exponential-phase cells were harvested, fixed with 2.5% glutaraldehyde followed with postfixation in 1% osmium tetroxide and then 1% aueous uranyl acetate. Next, the samples were dehydrated and embedded in Epon 812. The thin sections were stained with uranyl acetate and lead citrate. Thirty cells of each strain with nearly equatorial cut surfaces were measured for the evaluation of cell wall thickness, and results were expressed as mean value± standard deviation (SD).

2.9. Pulsed-field gel electrophoresis (PFGE)

PFGE was performed with CHEF MAPPER (Bio-Rad, Hercules, CA) as described by Yoshida et al.[19] The separated DNA fragments digested with the enzyme SmaI were photographed after being stained with ethidium bromide. Strains were considered identical when they shared same number and sizes of fragments. The strains varied with only two or three bands were considered closely related.[20]

2.10. Statistical analysis

The statistical significance of the data was evaluated by student's *t* test in this study, P value < 0.05 was considered statistically significant.

3. Results

3.1. Antimicrobial susceptibilities

All isolates were subjected to up to 19 antimicrobics, including vancomycin (VAN) and teicoplanin (TEC). Table 1 shows that all strains isolated from the same patient through the therapeutic process displayed distinct antibiotic susceptibility patterns. All isolates showed stable resistance to Penicillin, Oxacillin, Cefoxitin, Cefazolin, Cefuroxime Tobramycin, Gentamicin, and Levofloxacin, while strain 5944 exhibited vancomycin intermediated-resistance with MIC of 8ug/ml which meet the criteria established by the Clinical and Laboratory Standards Institute (CLSI) in 2013 (Figure 2A). The antibiotic susceptibility pattern was further identified by PAP/AUC. As shown in Figure 2B, the ratio of the AUC of the 5944 to the AUC of Mu3 was >1.3. The ratio of clinical strains, 5916, 5965, 5966, 5967, 5968 was 0.90 to 1.3, was defined as hVISA. The ratio of 5135, 5247, 5493 were <0.90, was defined as VSSA.

3.2. Molecular characteristics and the presence of genes associated with VISA

SCCmec typing revealed that all 9 isolates contained SCCmec type III, and displayed *mecA* gene positive. MLST typing, Spa typing and agr grouping test indicated that all 9 isolates belonged to

Table 1
Comparison of antibiotic susceptibilities

Antimicrobial agents	5135	5247	5493	5916	5944	5965	5966	5967	5968
Tigecycline	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chloramphenicol	8	8	8	8	8	8	8	8	8
Clindamycin	0.5	0.5	0.5	0.5	256	0.5	256	256	256
Erythromycin	0.5	0.5	1	0.5	256	0.5	256	256	256
fosfomycin	16	32	32	16	32	32	16	32	32
Teicoplanin	2	2	2	2	2	2	2	2	2
Linezolid	2	2	2	2	2	2	2	2	2
Rifampin	64	64	64	64	64	64	64	64	64
Teicoplanin	2	2	2	2	2	2	2	2	2
TEC-Etest	3	2	2	3	3	3	3	3	2
Vancomycin	1	1	1	1	8	1	1	1	1
VAN-Etest	1	1	1	1	8	1	1	1.5	1
Trimethoprim/sulfamethoxazole	1/19	1/19	1/19	1/19	2/38	1/19	2/38	1/19	1/19
Tobramycin	256	256	256	256	256	256	256	256	256
Gentamicin	256	256	256	256	256	256	256	256	256
Levofloxacin	256	256	256	256	256	256	256	256	256
Penicillin	256	256	256	256	256	256	256	256	256
Oxacillin	256	256	256	256	256	256	256	256	256
Cefoxitin	256	256	256	256	256	256	256	256	256
Cefazolin	256	256	256	256	256	256	256	256	256
Cefuroxime	256	256	256	256	256	256	256	256	256

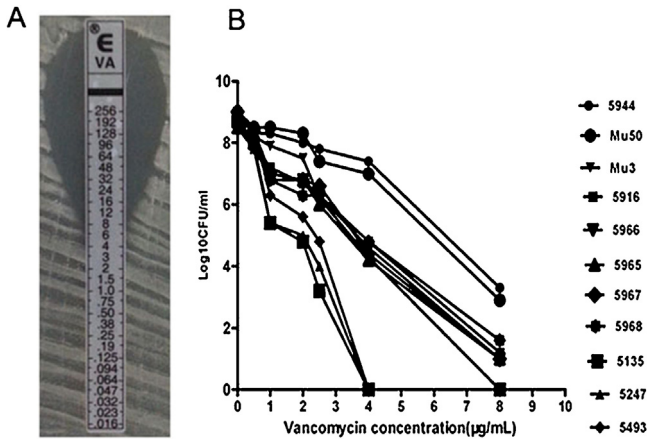

Figure 2. Population analysis profile curves and vancomycin MIC by E-test. (A) Strain 5944 was grown overnight in brain heart infusion medium and subjected to vancomycin MIC by E-test. (B) Population analysis profile of 9 isolates in this study and 2 standard strains (VISA strain Mu50 and hVISA strain Mu3). The curves are representative of at least 3 experiments with each strain.

ST239-t030-agr-1. None of those three genes, VanA, VanB and VanC was found in Strain 5944.

3.3. VISA growth rate and hemolytic activity

In addition to the test of antibiotic susceptibility, we got insight into the change of morphological features of strain 5944. The size of colonies formed on blood agar plates by strain 5944 (right panel) was much smaller than that of 5135, a VSSA strain identified with PAP/AUC (left panel), indicating a slower growth of the former (Figure 3). Comparison of hemolytic zones induced by strain 5944 and 5135 showed the marginally significant decrease of hemolytic activity of VISA strain 5944 (Figure 3).

3.4. Susceptibility to Triton X-100 induced autolysis

Reduced susceptibility to Triton X-100-induced autolysis was found in strain 5944 (Figure 4).

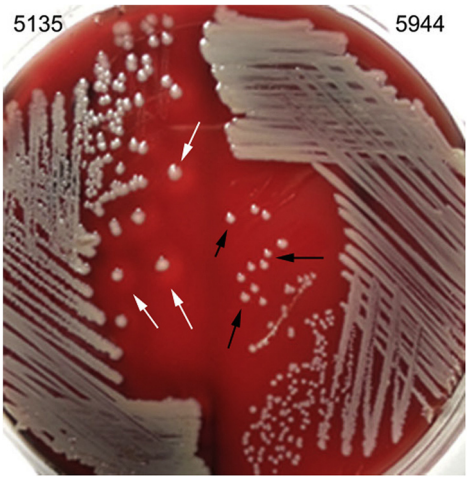

Figure 3. Low growth rate and reduced hemolytic activity. strain 5135and strain 5944 were plated onto blood agar plates and incubated for 24 h at 37 °C. Strain 5135 had significantly larger colonies than those of strain 5944 with invisible hemolytic zone. The single colony of bacteria was marked with hollow arrow (5135) or solid arrow (5944).

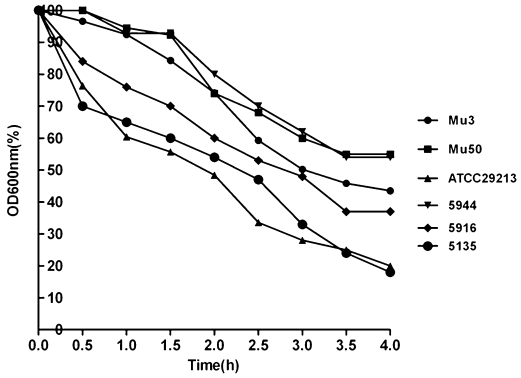

Figure 4. Triton X-100 stimulated autolysis assay. Absorbance is represented as the percentage of the absorbance at OD_{600} nm relative to that at time zero for each sample. Strain 5944 exhibited decreased autolysis, the same as VISA control strain Mu50. The test was repeated 3 times, and 1 representative was indicated.

3.5. Cell wall morphologies

A total of 4 *Staphylococcus aureus* strains, a standard VSSA strain ATCC 25923, three clinical isolates 5135 (VSSA), 5916 (hVISA) and 5944(VISA) were subjected to morphometric study using transmission electron microscopy. Figure 5A shows transmission electron micrography of representative strains. As is evident in Figure 5B, clinical VISA strain 5944 had significantly thicker cell walls than 5916, 5139 and the control strain ATCC 25923 ($p<0.05$).

3.6. PFGE patterns

All 9 clinical isolates were subjected to PFGE analysis. The same DNA fragments were found in each electrophoresis channel (Fig. 6).

4. Discussion

In the present study, we report a case of VISA isolated from a 51-year-old male patient in Tongji Hospital in China. It is the first report of VISA in Central-Southern China. Furthermore, it is noteworthy that the patient was never treated with vancomycin.

The identified VISA strain, strain 5944 was subjected to *SCCmec* typing and *mecA* detection, showing that strain 5944 was *SCCmec* type III and *mecA* positive, a predominant strain in China, which is linked with the previous report.[9] *SCCmec* type III was a prevalent MRSA clone in European countries, Australia, Thailand and so on,[21] which is different from the conclusion from Katayama and Buntaran's studies that *SCCmec* II predominated in the Asian-pacific region.[22]

To further understand the molecular epidemiological features of this VISA isolate, we sought to characterize it by MLST typing, Spa typing and agr detection. Our data showed that strain 5944 in this study belonged to MLST type ST239 and spa type t030, a predominant strain in China, and even in the whole of Asia.[23,24] Agr detection demonstrated that 5944 belonged to agr type 1, one of the most common MRSA clones in central-south China.[10] However, Moise-Broder's work suggested that agr type 2 was more likely to develop glycopeptide resistance than agr type 1 strains.[25] We think this different characteristic might be due to the regional difference because all samples in Moise's study were recovered from the United States and Japan. We did not harvest enough samples to determine whether agr typing could be an independent predictor of vancomycin treatment failure in patients with MRSA infection as shown in the previous study.[25] Our result of VISA genotyping was the same as the previous report that was the first report of VISA from the Third Military Medical University, Chongqing, China.[26]

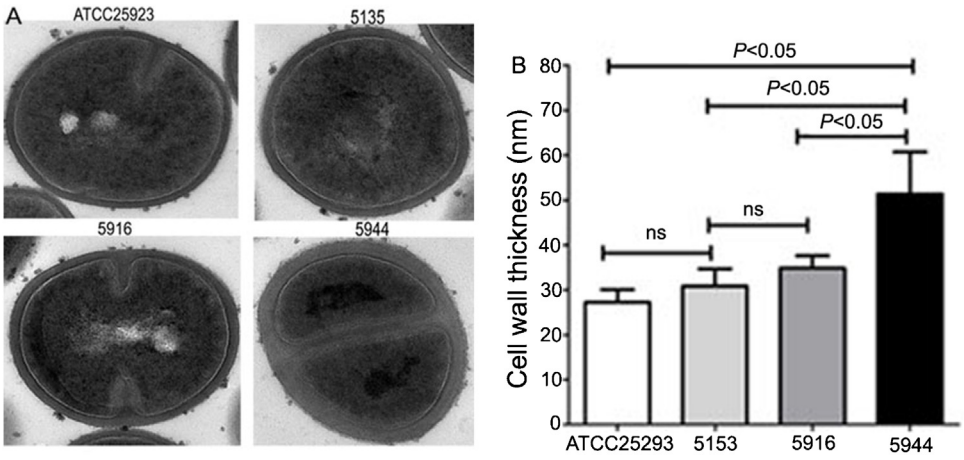

Figure 5. Transmission electron microscopy of representative strains. (A) Transmission electron microscopy of ATCC 25923, 5135, 5916 and 5944. Magnification, ×25,000. (B) Comparison of cell wall thickness between the strains ATCC 25923, 5135, 5916 and 5944. The data are expressed as the mean±SD of 30 cells of each strain for determination and were evaluated by the Student's t test. *P* value less than 0.05 is considered statistically significant. Ns = not significant.

Common features of glycopeptides intermediate-resistant staphylococcus aureus (GISA) include cell wall-thickening, reduced autolysis, decreased growth rate and hemolysis.[27–29] In the present study, both reduced autolysis and decreasing of growth rate and hemolysis characterize this VISA isolate in Tongji hospital. The significant change of the cell wall of strain 5944 was found, versus the standard strain Mu3. The previous study demonstrated that cell wall thickening is responsible for both vancomycin resistance and teicoplanin in staphylococcus aureus,[26] which might be attributed to same type of antibiotics both of them were attributed to. However, the strain 5944 in this study remained sensitive to teicoplanin.

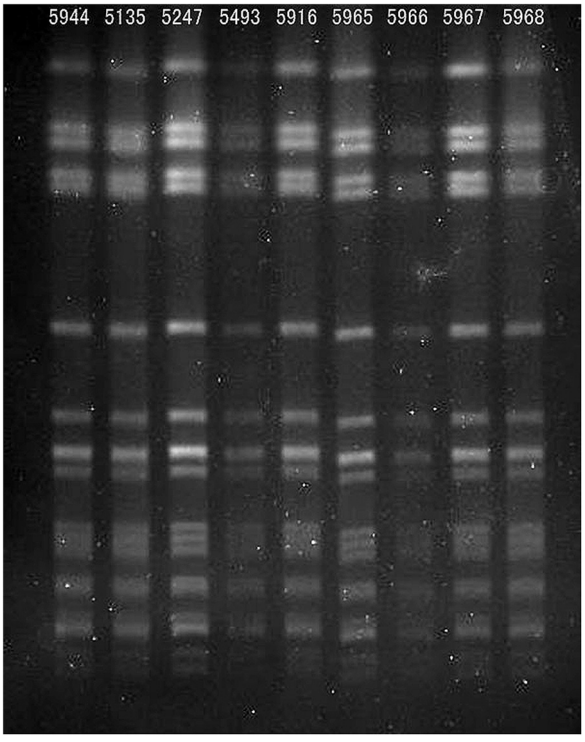

Figure 6. PFGE banding patterns of 9 clinical isolates. All isolates exhibited the exact same PFGE pattern.

To determine the mechanism of acquirement of reduced vancomycin susceptibility of strain 5944, we also detected the Van gene which was implicated in generation of Vancomycin resistance.[28,30] There are increasing documents showing that *S.aureus* could acquire vancomycin resistance by transferring Van genes including VanA, VanB and VanC,[31–33] which was even accomplished in the laboratory.[34] But neither VanA, VanB nor VanC was found in VISA isolate.

Since Vancomycin is regarded as one of the mainstays of treatment for MRSA infection, the use of Vancomycin in this hospital is common, about 4.49 DDDs/100 admissions, which led to a high prevalence of hVISA in MRSA isolates.[10] In the present study, the patient with Esophageal cancer twice underwent surgeries which might have induced poor physical health. Scott K Fridkin *et al*'s study indicated that recurrent MRSA infection and certain underlying illnesses might increase the risk of development of VISA.[35] It seemed that in this case, the serious underlying illness and high chance of exposure to MRSA and hVISA colonization might also contribute to VISA infection. But we could not determine the reason behind it. This is the first report of VISA Isolated from a patient who never received Vancomycin treatment.

By far, the overwhelming majority of S.aureus isolates still are Vancomycin-susceptible, having an MIC in the range of 0.5-2ug/ml. However, we must watch out for emergence of VISA or VRSA among inpatients.

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Conflict of interest statement: We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

We wish to confirm that there are no known conflicts of interest associated with this publication.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from international journal of infectious disease.

References

1. Projan SJ. Antibiotic resistance in staphylococci. In: Fischetti VA, Novick RP, Ferreti JJ, Portnoy DA, Rood JI, editors. *Gram-Positive Pathogens*. Washington, DC: American Society for Microbiology; 2000. p. 463–70.
2. Aires de Sousa M, Crisostomo MI, Sanches IS, Wu JS, Fuzhong J, Tomasz A, et al. Frequent recovery of a single clonal type of multidrug-resistant Staphylococcus aureus from patients in two hospitals in Taiwan and China. *J Clin Microbiol* 2003;**41**:159–63.
3. Lee K, Chang CL, Lee NY, Kim HS, Hong KS, Cho HC. Korean Nationwide Surveillance of Antimicrobial Resistance of Bacteria in 1998. *Yonsei Med J* 2000;**41**:497–506.
4. Voss A, Doebbeling BN. The worldwide prevalence of methicillin-resistant Staphylococcus aureus. *Int J Antimicrob Agents* 1995;**5**:101–6.
5. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. *Lancet* 1997;**350**:1670–3.
6. Ho CM, Hsueh PR, Liu CY, Lee SY, Chiueh TS, Shyr JM, et al. Prevalence and accessory gene regulator (agr) analysis of vancomycin-intermediate Staphylococcus aureus among methicillin-resistant isolates in Taiwan–SMART program, 2003. *Eur J Clin Microbiol Infect Dis* 2010;**29**:383–9.
7. Lu JJ, Lee SY, Hwa SY, Yang AH. Septic arthritis caused by vancomycin-intermediate Staphylococcus aureus. *J Clin Microbiol* 2005;**43**:4156–8.
8. Trakulsomboon S, Danchaivijitr S, Rongrungruang Y, Dhiraputra C, Susaemgrat W, Ito T, et al. First report of methicillin-resistant Staphylococcus aureus with reduced susceptibility to vancomycin in Thailand. *J Clin Microbiol* 2001; **39**:591–5.
9. Sun W, Chen H, Liu Y, Zhao C, Nichols WW, Chen M, et al. Prevalence and characterization of heterogeneous vancomycin-intermediate Staphylococcus aureus isolates from 14 cities in China. *Antimicrob Agents Chemother* 2009;**53**: 3642–9.
10. Liu C, Chen ZJ, Sun Z, Feng X, Zou M, Cao W, et al. Molecular characteristics and virulence factors in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate Staphylococcus aureus from central-southern China. *J Microbiol Immunol Infect* 2014.
11. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. *J Antimicrob Chemother* 2001;**47**:399–403.
12. Milheirico C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in Staphylococcus aureus. *Antimicrob Agents Chemother* 2007;**51**:3374–7.
13. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. *J Clin Microbiol* 2000;**38**:1008–15.
14. Wang WY, Lee SY, Chiueh TS, Lu JJ. Molecular and phenotypic characteristics of methicillin-resistant and vancomycin-intermediate staphylococcus aureus isolates from patients with septic arthritis. *J Clin Microbiol* 2009;**47**: 3617–23.
15. Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol* 2003;**41**:5442–8.
16. Lu JJ, Perng CL, Chiueh TS, Lee SY, Chen CH, Chang FY, et al. Detection and typing of vancomycin-resistance genes of enterococci from clinical and nosocomial surveillance specimens by multiplex PCR. *Epidemiol Infect* 2001;**126**: 357–63.
17. Sieradzki K, Tomasz A. Alterations of cell wall structure and metabolism accompany reduced susceptibility to vancomycin in an isogenic series of clinical isolates of Staphylococcus aureus. *J Bacteriol* 2003;**185**:7103–10.
18. Cui L, Murakami H, Kuwahara-Arai K, Hanaki H, Hiramatsu K. Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by Staphylococcus aureus Mu50. *Antimicrob Agents Chemother* 2000;**44**:2276–85.
19. Yoshida T, Kondo N, Hanifah YA, Hiramatsu K. Combined use of ribotyping, PFGE typing and IS431 typing in the discrimination of nosocomial strains of methicillin-resistant Staphylococcus aureus. *Microbiol Immunol* 1997;**41**: 687–95.
20. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**:2233–9.
21. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. *Antimicrob Agents Chemother* 2000;**44**:1549–55.
22. Buntaran L, Hatta M, Sultan AR, Dwiyanti R, Sabir M. Sccmec type II gene is common among clinical isolates of methicillin-resistant Staphylococcus aureus in Jakarta, Indonesia. *BMC Res Notes* 2013;**6**:110.
23. Abimanyu N, Murugesan S, Krishnan P. Emergence of methicillin-resistant Staphylococcus aureus clones in a Chinese tertiary care hospital over a 15-year period. *Antimicrob Agents Chemother* 2010;**54**:1842–7.
24. Chen H, Liu Y, Jiang X, Chen M, Wang H. Rapid change of methicillin-resistant Staphylococcus aureus clones in a Chinese tertiary care hospital over a 15-year period. *Antimicrob Agents Chemother* 2010;**54**:1842–7.
25. Moise-Broder PA, Sakoulas G, Eliopoulos GM, Schentag JJ, Forrest A, Moellering Jr RC. Accessory gene regulator group II polymorphism in methicillin-resistant Staphylococcus aureus is predictive of failure of vancomycin therapy. *Clin Infect Dis* 2004;**38**:1700–5.
26. Zhang X, Hu Q, Yuan W, Shang W, Cheng H, Yuan J, et al. First report of a sequence type 239 vancomycin-intermediate Staphylococcus aureus isolate in Mainland China. *Diagn Microbiol Infect Dis* 2013;**77**:64–8.
27. Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, et al. Cell wall thickening is a common feature of vancomycin resistance in Staphylococcus aureus. *J Clin Microbiol* 2003;**41**:5–14.
28. Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev* 2010;**23**:99–139.
29. Marlowe EM, Cohen MD, Hindler JF, Ward KW, Bruckner DA. Practical strategies for detecting and confirming vancomycin-intermediate Staphylococcus aureus: a tertiary-care hospital laboratory's experience. *J Clin Microbiol* 2001;**39**:2637–9.
30. Peleg AY, Monga D, Pillai S, Mylonakis E, Moellering Jr RC, Eliopoulos GM. Reduced susceptibility to vancomycin influences pathogenicity in Staphylococcus aureus infection. *J Infect Dis* 2009;**199**:532–6.
31. Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in Enterococcus faecium. *N Engl J Med* 1988; **319**:157–61.
32. Quintiliani Jr R, Evers S, Courvalin P. The vanB gene confers various levels of self-transferable resistance to vancomycin in enterococci. *J Infect Dis* 1993;**167**: 1220–3.
33. Uttley AH, George RC, Naidoo J, Woodford N, Johnson AP, Collins CH, et al. High-level vancomycin-resistant enterococci causing hospital infections. *Epidemiol Infect* 1989;**103**:173–81.
34. Dubberke ER, Reske KA, Noble-Wang J, Thompson A, Killgore G, Mayfield J, et al. Prevalence of Clostridium difficile environmental contamination and strain variability in multiple health care facilities. *Am J Infect Control* 2007; **35**:315–8.
35. Fridkin SK, Hageman J, McDougal LK, Mohammed J, Jarvis WR, Perl TM, et al. Epidemiological and microbiological characterization of infections caused by Staphylococcus aureus with reduced susceptibility to vancomycin, United States, 1997–2001. *Clin Infect Dis* 2003;**36**:429–39.