CLINICAL ARTICLES

Bacteremia Caused by Staphylococci with Inducible Vancomycin Heteroresistance

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The clinical significance of bacteremia due to vancomycin-heteroresistant staphylococci and a rapid laboratory screening method were examined; 203 strains of staphylococci isolated from patients with clinically significant bacteremia were screened by the disk-agar method with use of vancomycin-salt agar to demonstrate satellitism around an aztreonam disk as well as by conventional population screening. Eighteen isolates (three *Staphylococcus aureus* and 15 coagulase-negative staphylococci) were shown to be heteroresistant to vancomycin. A case-control clinical study showed that the interval between admission and bacteremia, admission to the intensive care unit, prior use of vancomycin and/or β-lactams, and isolation of methicillin-resistant staphylococci were significantly more common among patients with bacteremia due to staphylococci with heteroresistance to vancomycin; these patients had an overall mortality of 44.4%. The use of vancomycin and admission to the intensive care unit were independently significant risk factors on multivariate analysis. Vancomycin heteroresistance is inducible by salt and β-lactams. Indiscriminate sequential use of β-lactams and glycopeptides may facilitate the emergence of glycopeptide resistance.

Glycopeptides have long been the last resort in the treatment of serious infections due to multiresistant gram-positive organisms. The alarming emergence of *Staphylococcus aureus* with intermediate resistance to vancomycin [1–3] and *S. aureus* with heteroresistance to vancomycin [4] was recently reported, and therapeutic failures with glycopeptides have been observed in treatment of infections due to *S. aureus* with intermediate vancomycin resistance [1, 3]. However, routine laboratory detection of *S. aureus* with intermediate resistance and with heteroresistance to vancomycin is difficult and not yet standardized. The terminology for staphylococci with reduced susceptibility to glycopeptides still needs refinement, as different break points are used by different investigators to define resistance.

See editorial response by Moellering on pages 768–70.

The exact clinical significance of vancomycin-heteroresistant staphylococci is still unknown. Even less is known about this phenomenon among coagulase-negative staphylococci (CNS), which have emerged as important nosocomial pathogens in recent years. Previous in vitro study of *Staphylococcus haemolyticus* [5] has demonstrated that vancomycin resistance is detectable only among imipenem-resistant strains and that the phenotypic resistance is inducible by imipenem. The addition of 2% NaCl to Mueller-Hinton culture broth was necessary for the growth of resistant clones in the presence of high concentrations of vancomycin. We made use of such growth characteristics in the design of a disk-agar screening method for the rapid screening of salt- and aztreonam-inducible vancomycin heteroresistance among staphylococcal isolates obtained from blood. The clinical characteristics and outcome of these patients are reported in a case-control study.

Materials and Methods

Strains of staphylococci were isolated from blood between 1 July 1997 to 30 June 1998 at the Department of Microbiology, Queen Mary Hospital, a 1,400-bed tertiary care and university teaching hospital. All *S. aureus* isolated during this period were included in the study. CNS were selected only if the same strain was isolated on culture of blood from two different sites from the same febrile patient or if the isolate from blood was identical to that obtained by culture from central venous catheters and determined to be positive by the roll plate method with >15 cfu [6]. There were no duplicate isolates from the same patient. Staphylococci were identified by conventional biochemical tests [7], including the Staphaurex latex agglutination kit (Murex Biotech, Dartford, UK) and tube coagulase

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Table 1. Comparison of results of testing for vancomycin resistance by using population screening and disk-agar methods.

Classification of isolates	No. tested	No. initially positive* by population screening	No. initially positive* by disk-agar method	No. with confirmed heteroresistance to vancomycin†
Methicillin-susceptible *Staphylococcus aureus*	112	3	0	0
Methicillin-resistant *S. aureus*	52	21	5	3
Methicillin-susceptible coagulase-negative staphylococci	8	0	0	0
Methicillin-resistant coagulase-negative staphylococci	31	28	21	15
Total	203	52	26	18

* Strains that are initially positive had subpopulations that grew on the screening plates.
† Strains are referred to as having confirmed heteroresistance to vancomycin if the vancomycin MIC of these subpopulations was ≥ 8 μg/mL.

test; the identity was further confirmed by the Vitek gram-positive card (bioMérieux Vitek, Hazelwood, MO). Routine antibiotic sensitivity testing was done by the disk diffusion method (BBL Sensi-Disc; Becton Dickinson, Cockeysville, MD) according to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [8, 9]. A strain of vancomycin-resistant *S. haemolyticus* and *S. aureus* ATCC 29213 were used as controls throughout the study.

Screening for potential vancomycin resistance by population screening. *S. aureus* strains were screened for potential vancomycin resistance as previously described [4]. Ten microliters of a bacterial suspension made from an overnight agar culture adjusted to McFarland standard 0.5 was spread on brain-heart infusion agar (Oxoid, Basingstoke, UK) with 4 μg/mL vancomycin and incubated at 37°C for 48 h. The presence of growth (initial positive, table 1) as well as the number of colonies were noted at 48 h. The vancomycin MICs for these subclones were first determined by the Etest (AB BIODISK, Solna, Sweden) and then confirmed by the broth macrodilution method if the vancomycin MIC was ≥ 4 μg/mL by Etest.

Screening for potential vancomycin resistance by the disk-agar method. Vancomycin-salt agar was prepared by incorporating 4 μg/mL vancomycin and 4% NaCl into Mueller-Hinton agar (Oxoid). Agar was poured into 85-mm Petri dishes, with a 4-mm depth of agar. An overnight blood agar plate culture of the isolates was used to make a bacterial suspension of McFarland 1 turbidity in 0.9% saline. The agar was evenly inoculated by a sterile cotton swab dipped into the suspension and pressed firmly on the inside of the tube to remove excess inoculum from the swab. A 30-μg aztreonam disk (BBL Sensi-Disc; Becton Dickinson) was placed on the agar surface 5 minutes afterwards. The plates were incubated at 37°C for 48 hours. The largest single colony on the vancomycin-salt agar demonstrating satellitism around the aztreonam disk was picked out for determination of vancomycin MIC by the Etest, followed by confirmation with the macrobroth dilution method if the vancomycin MIC by the Etest was ≥ 4 μg/mL.

Confirmation of identity and MIC determination. Colonies that grew on vancomycin-containing agar were retested biochemically to confirm their identity. Final determinations of vancomycin MICs were done by the broth macrodilution method [10] and Etest with and without the addition of NaCl to the medium (2% and 4% NaCl to the Mueller-Hinton broth and agar, respectively). For the broth macrodilution method, the inoculum was 5×10^5 cfu/mL (range, $3–7 \times 10^5$ cfu/mL) and was confirmed by back titration and plating on blood agar plates. The serial concentrations of vancomycin in the Mueller-Hinton broth were confirmed by use of the TDX Analyzer (Abbott Laboratories, North Chicago, IL) prior to the test. Strains that were identified as *S. haemolyticus* were excluded from the study if they showed homogenous resistance to vancomycin by disk diffusion testing.

Case-control study. Patients with bacteremia due to staphylococci with heteroresistance to vancomycin were defined as cases. Age- and sex-matched controls were taken from patients with staphylococcal bacteremia in the same period but whose isolates did not demonstrate vancomycin heteroresistance. Ages of control patients were ± 3 years of the respective case-patient for adults or ± 1 month for infants and neonates. Demographic and clinical information for the cases and controls were compared to identify characteristics associated with bacteremia due to staphylococci with heteroresistance to vancomycin. The χ^2 test was used for statistical analysis, and $P < .05$ was considered statistically significant. Variables that were statistically significant were further tested by multivariate logistic analysis by use of the backward elimination method.

Table 2. Microbiological characteristics of staphylococci demonstrating vancomycin heteroresistance.

		MIC of parent strain at 24 h (μg/mL)		MIC of subclone after selection at 24 h (μg/mL)		Other antibiotic susceptibility				
Case no.	Strain identification	Without NaCl	With 2% NaCl	Without NaCl	With 2% NaCl	Cm	Em	FA	Gm	TMP-SMZ
70	*Staphylococcus aureus*	1	1	8	8	R	R	S	R	S
231	*S. aureus*	1	2	8	16	R	R	S	R	S
500	*S. aureus*	2	2	8	8	R	R	S	R	S
17	*Staphylococcus epidermidis*	2	8	8	32	R	R	S	R	R
37	*S. epidermidis*	2	2	16	32	R	R	R	R	R
148	*S. epidermidis*	2	4	8	16	R	R	S	S	S
412	*S. epidermidis*	2	2	8	8	R	R	R	R	R
425	*S. epidermidis*	2	2	8	16	R	R	S	S	R
431	*S. epidermidis*	2	2	8	8	R	R	R	R	R
52	*Staphylococcus capitis*	1	1	16	64	R	R	S	R	S
106	*S. capitis*	2	4	16	16	R	R	S	R	S
159	*S. capitis*	2	2	8	16	R	R	R	S	S
446	*Staphylococcus haemolyticus*	4	4	8	32	R	R	S	R	R
183	*Staphylococcus auricularis*	2	4	16	16	R	R	R	R	S
427	*Staphylococcus simulans*	2	4	16	64	R	R	S	S	S
428	*Staphylococcus warneri*	2	2	8	16	R	R	S	S	S
56	Coagulase-negative staphylococci	1	2	8	16	R	R	R	S	S
422	Coagulase-negative staphylococci	2	4	8	8	R	R	S	R	R

NOTE. All isolates were methicillin-resistant. Cm = clindamycin; Em = erythromycin; FA = fusidic acid; Gm = gentamicin; R = resistant; S = susceptible; TMP-SMZ = trimethoprim-sulfamethoxazole. Susceptibility to antibiotics other than vancomycin was determined by disk diffusion test and vancomycin MIC was determined by the macrobroth dilution according to National Committee for Clinical Laboratory Standards methodology.

Definitions. Methicillin resistance was defined as an oxacillin MIC of $\geq$4 μg/mL as tested by use of the NCCLS method [8]. An isolate was defined as having intermediate vancomycin resistance if the vancomycin MIC was >4 but <32 μg/mL, whereas vancomycin resistance was defined as an MIC of $\geq$32 μg/mL [8]. Heteroresistance to vancomycin was defined as occurring if a strain produced a subclone(s) at a frequency of $\geq 10^{-6}$ colonies with vancomycin MIC of $\geq$8 μg/mL on selection by the population screening, with the stability of the strain persisting beyond 9 days in an antibiotic-free medium [4]. An episode of bacteremia was considered nosocomial in origin if the time between admission and the date of positive blood culture was >2 days [11].

Results

Two hundred three strains of staphylococci from blood were retrieved for screening, comprising 112 methicillin-sensitive *S. aureus,* 52 methicillin-resistant *S. aureus,* 8 methicillin-sensitive CNS, and 31 methicillin-resistant CNS (table 1). Eighteen strains (table 2) demonstrated heteroresistance to vancomycin after screening by the disk-agar method, including 3 strains of methicillin-resistant *S. aureus* (5.8% prevalence) and 15 strains of methicillin-resistant CNS (48.4% prevalence). Six of these 15 CNS were identified as *Staphylococcus epidermidis* and three were *Staphylococcus capitis.* None of the methicillin-sensitive isolates yielded staphylococci with heteroresistance to vancomycin subclones after screening by either method. The antibiograms of the staphylococci with heteroresistance to vancomycin were similar to those of other methicillin-resistant staphylococci isolated in this hospital in that most were multiresistant to other antibiotics, including erythromycin (100% resistance), clindamycin (100% resistance), and gentamicin (66.7% resistance), but remained susceptible to fusidic acid (33.3% resistance).

The parent strains of the 18 staphylococcal isolates with heteroresistance to vancomycin were all sensitive to vancomycin (vancomycin MIC of $\leq$4 μg/mL) by conventional antibiotic susceptibility testing (table 2). Subclones selected out by population screening or the disk-agar method showed 2- to

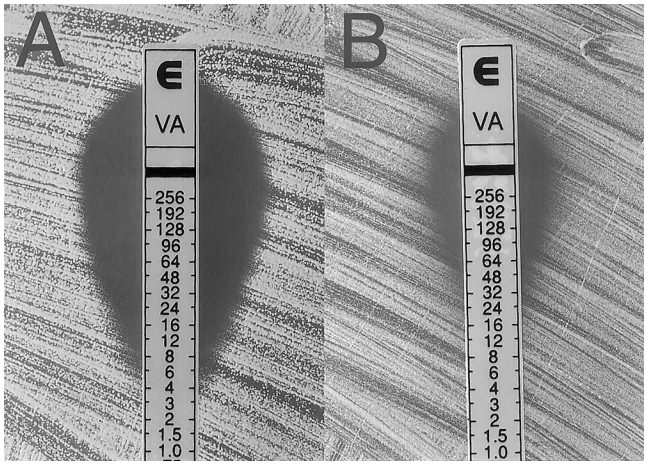

Figure 1. Vancomycin MIC of *Staphylococcus capitis* (case no. 159) determined by Etest. *A,* Tested on Mueller-Hinton agar; *B,* tested on Mueller-Hinton agar with the addition of 4% NaCl.

16-fold increases in vancomycin MICs as determined by standard testing methods. The majority of subclones had vancomycin MICs four- to eightfold higher than those of the parent strains.

Increasing the time of incubation to 48 hours resulted in a twofold rise in vancomycin MIC for a small number of parent strains (four) and subclones (six) (data not shown). In contrast, the addition of salt to the testing media showed a more prominent effect on vancomycin MIC: Eight of the parent strains and 11 of the subclones had two- to fourfold increases. The effect of NaCl on vancomycin MIC is figuratively illustrated by figure 1. Addition of 4% NaCl to Mueller-Hinton agar with 4 μg/mL vancomycin also markedly enhanced the phenomenon of satellitism around the aztreonam disk (figure 2).

The clinical characteristics of the cases are summarized in table 3. Eleven of the patients were admitted to the intensive care unit during or at least 2 days before the date of bacteremia. Cultures of blood from all case-patients yielded methicillin-resistant staphylococci, and the episodes of bacteremia were nosocomial in origin. Demographic and clinical data for the cases and controls are compared in table 4. The ratio of males to females was 13:5; the median age was 47.5 years. The overall mortality rate of the case-patients was 44.4%, compared with 10% in the control group. For seven of the cases, the infective foci originated from an infected intravascular catheter. By use of univariate analysis, bacteremia due to staphylococci with heteroresistance to vancomycin was associated with admission to the intensive care unit ($P < .005$), nosocomial bacteremia ($P < .005$), administration of vancomycin ($P < .001$) and/or other β-lactam antibiotics ($P < .05$) within 1 week of bacteremia, and methicillin-resistant staphylococcal bacteremia ($P < .05$). However, only the use of vancomycin (relative risk = 9.45) and admission to the intensive care unit (relative risk = 3.47) were statistically significant ($P < .05$) when these variables were analyzed by multivariate logistic analysis.

Discussion

Glycopeptide resistance among gram-positive bacteria has threatened the clinical usefulness of vancomycin and teicoplanin. Two of the four reported clinical cases of infection due to *S. aureus* with intermediate vancomycin resistance were associated with failure of treatment with a glycopeptide [1, 3]. A thorough understanding of the terminology, laboratory detection, and clinical significance is therefore essential to develop a rapid and sensitive method for screening for *S. aureus* with intermediate vancomycin resistance. The terminology of staphylococci with reduced susceptibility to glycopeptides is still not standardized. With the currently accepted NCCLS methodology and break points [8], no true vancomycin resistance has been found among clinical isolates [13]. Heteroresistance to vancomycin was described among 5%–26% of methicillin-resistant *S. aureus* isolates in Japanese hospitals [4].

The term "heteroresistance" may not be a satisfactory term to encompass all staphylococci containing subpopulations with elevated vancomycin MICs. First, the vancomycin MIC used to define vancomycin-resistant *S. aureus* and *S. aureus* with heteroresistance to vancomycin were different in different studies. Whereas the NCCLS break point for vancomycin resistance is $\geq$32 μg/mL, a vancomycin MIC of $\geq$8 μg/mL was used in a subsequent study [4]. Indeed, in the latter report of 35 *S. aureus* with heteroresistance to vancomycin, the highest vancomycin MIC of the "resistant" subclones was 7 μg/mL, which is, strictly speaking, intermediate vancomycin heteroresistance. In our study, the vancomycin MICs of the subclones were 8–16

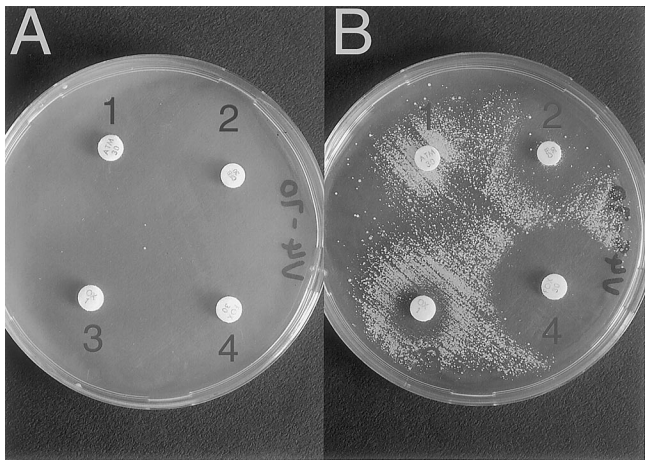

Figure 2. Satellitism of *Staphylococcus aureus* (case no. 70) around β-lactam antibiotic disks on Mueller-Hinton agar plates with 4 μg/mL vancomycin. *A,* Agar plate without addition of NaCl; *B,* agar plate with the addition of 4% NaCl. 1 = aztreonam; 2 = ceftibuten; 3 = oxacillin; 4 = cefoxitin.

Table 3. Clinical characteristics of patients with bacteremia due to vancomycin-heteroresistant staphylococci.

Case no.	Sex	Age (years)	Underlying disease	Infective focus	Antimicrobial agent(s) administered before or during blood culture*	Outcome
70	M	78	Chronic renal failure, receiving chronic ambulatory peritoneal dialysis	Sepsis and acute pyogenic arthritis of the right shoulder	None	Died despite Vm and Rif
231	F	53	Mycosis fungoides, undergoing chemotherapy	Neutropenic sepsis	Vm, Imi, Amik, Flu	Died despite continuing therapy
500	M	77	Carcinoma of stomach; given one course of regional chemotherapy before admission	Central venous catheter	Vm	Afebrile after vancomycin and oral fusidic acid
17	M	65	Subarachnoid hemorrhage, hydrocephalus, coma; ventriculoperitoneal shunt in situ	Central venous catheter	Ctri	Recovered after removal of catheter
37	M	34	Carcinoma of colon, postcolectomy, short gut syndrome after resection for radiation enteritis	Central venous catheter	None	Recovered after removal of Broviac catheter
148	F	0.5	Prematurity, necrotizing enterocolitis, anastomotic leakage	Sepsis, peritonitis	Czid, Vm	Died despite addition of Mtz
412	F	44	Acute traumatic subdural hematoma, fractured cervical spine, quadriplegia	Central venous catheter	Pip/Taz, Gm	Recovered after removal of central venous catheter
425	F	59	Traumatic fracture of the skull and coma after craniectomy	Central venous catheter	Vm, Ctri, Flu	Recovered after removal of central venous catheter and addition of fusidic acid
431	M	0.45	Prematurity, short gut syndrome after resection for necrotizing enterocolitis	Sepsis	Czid, Clox, Mtz	Died despite switching from Clox to Vm
52	M	0.2	Gut resection for malrotation and volvulus	Central venous catheter	Czid, Mtz	Recovered after removal of Broviac and addition of Vm
106	M	64	Subarachnoid hemorrhage, hydrocephalus, coma; ventriculoperitoneal shunt in situ	Central venous catheter	Ctri	Recovered after removal of central venous catheter
159	M	14 d	Prematurity	Sepsis	Vm	Recovered
446	M	17	Acute myeloid leukemia, undergoing chemotherapy	Neutropenic sepsis	Imi, Vm	Afebrile after WBC count returned to normal
183	M	23	Lymphoma, undergoing chemotherapy	Neutropenic sepsis	Imi, Vm	Died
427	M	62	Acute myeloid leukemia	Neutropenic sepsis	Cpfx	Died despite Vm, Czid, and Mtz
428	M	0.5	Meconium ileus and peritonitis, Hirschsprung's disease	Sepsis	Czid, Vm, Mtz	Died despite addition of Net
56	M	76	Hodgkin's lymphoma, undergoing chemotherapy	Neutropenic sepsis	Czid	Died despite Vm and Cpfx
422	F	51	Carcinoma of the lung, undergoing chemotherapy	Neutropenic sepsis	Meropenem	Died despite addition of Vm

NOTE. Amik = amikacin; Clox = cloxacillin; Cpfx = ciprofloxacin; Ctri = ceftriaxone; Czid = ceftazidime; F = female; Flu = fluconazole; Gm = gentamicin; Imi = imipenem; M = male; Mtz = metronidazole; Net = netilmicin; Pip = piperacillin; Rif = rifampin; Taz = tazobactam; Vm = vancomycin.
* Route of administration of all antibiotics is iv unless otherwise stated.

μg/mL as determined by use of standard methodology (24 hours of incubation in the absence of added salt; table 2). Higher vancomycin MICs in the range of resistance can be observed only after 48 hours of incubation and/or addition of salt to the testing media.

Second, the conventional concept of methicillin "heterore-

Table 4. Risk factors for bacteremia due to vancomycin-heteroresistant staphylococci.

	Cases (n = 18)	Controls (n = 30)	P*
Sex ratio (M:F)	13:5	19:11	NS
Age			
Median (y)	47.5	57.5	NS
Range	14 d–78 y	16 d–78 y	
Type of ward			
Adult and neonatal ICU	11	6	<.005†
Hematology and oncology	3	3	NS
General medical/surgical	4	21	<.001
Underlying diseases‡			
Fatal	—	—	NS
Ultimately fatal	9	12	NS
Nonfatal	9	18	NS
Interval between day of admission to bacteremia			
≤2 d	—	11	<.005
Median (d)	14.5	9.0	
Range (d)	3–160	0–64	
Vancomycin (iv) given within 1 w of bacteremia	8 (44.4%)§	1 (3.0%)	<.001†
β-lactam (iv) given within 1 w of bacteremia	13 (72.2%)§	12 (40.0%)	<.05
Bacteremia due to methicillin-resistant staphylococci	18 (100%)	19 (63.3%)	<.05
Bacteremia due to *Staphylococcus aureus*	3 (16.7%)	21 (70.0%)	<.001
Deaths	8 (44.4%)	3 (10.0%)	

NOTE. Data are no., no. (%), or specified unit. F = female; ICU = intensive care unit; M = male.

* By univariate analysis. NS = not significant.

‡ The nature of underlying illness was classified according to [12].

§ Six patients concomitantly received vancomycin and a β-lactam antibiotic.

† Also significant by multivariate logistic analysis ($P < .05$). Relative risk associated with admission to ICU = 9.45; relative risk associated with use of vancomycin = 3.47.

sistance" in *S. aureus* refers to the occurrence of phenotypic resistance at a low frequency (~1 in 10^6) [14]; subculturing these resistant subpopulations on antibiotic-free media generally does not result in the appearance of homogenous resistance. This is in contrast with our experience in that the subclones with elevated vancomycin MICs are often phenotypically homogenous. A more exact description should therefore be "staphylococci with subpopulations showing reduced susceptibility to vancomycin." A better and more precise definition awaits a consensus on these issues and more information on the genetic basis of these staphylococci.

Vancomycin "heteroresistance" nevertheless resembles methicillin heteroresistance in other respects. Staphylococcal resistance to methicillin is better demonstrated under conditions such as neutral to slightly alkaline media, lower incubation temperature, or the presence of an increased concentration of salt [15]. The role of these conditions in the induction of vancomycin resistance is less clear. Among *S. haemolyticus,* vancomycin-resistant subclones demonstrated a double-zone phenomenon around a 10-μg imipenem disk, presumably because of induction by β-lactam antibiotics [5]. Addition of 2% NaCl to the medium also enhanced the growth of these subclones in the presence of high concentrations of vancomycin. This is, however, the first time that these properties have been used for the detection of vancomycin resistance in *S. aureus.* The mechanism by which these two factors enhance the expression of heteroresistance to vancomycin is not known. However, vancomycin-resistant *S. aureus* mutants thus far selected have been shown to have markedly thickened cell walls and were able to bind vancomycin present in the media [16]. It has also been demonstrated that glycopeptide resistance in *S. aureus* is associated with increased production of penicillin-binding proteins, most notably penicillin-binding protein 2 [17]. The increased production of peptidoglycan cell wall material in turn binds a large amount of vancomycin, resulting in blockage of cell autolysis, depletion of vancomycin in the medium, and steric hindrance so that free vancomycin in the environment is not able to penetrate the cell wall to bind to the D-alanyl-D-alanine termini of new muropeptides [16]. As the phenotypic expression of methicillin resistance in staphylococci can also be induced by the presence of a higher salt concentration [18], this might provide clues for further study of the association between higher salinity and the expression of vancomycin resistance.

The disk-agar screening method was based on the above observations with vancomycin-salt agar together with the demonstration of satellitism around a 30-μg aztreonam disk. The agar screening method is at present the only available screening method for the detection of *S. aureus* with intermediate vancomycin resistance but not staphylococci with heteroresistance to vancomycin [12]. The disk-agar method therefore has the advantage of demonstrating both the presence of potential intermediately vancomycin-resistant staphylococci and staphylococci with heteroresistance to vancomycin on the same plate. Aztreonam was preferred over other β-lactam antibiotics because it has no inhibitory effect on gram-positive bacteria. A preliminary study of the disk-agar screen method was done with different β-lactams including penicillins, oxacillin, cephalosporins, and carbapenems (data not shown). Although satellitism can sometimes be seen around other β-lactam disks, the results were less consistent than they were for aztreonam, and some strains had a large zone of inhibition around the disk (e.g., around the cefoxitin disk in figure 2). Besides being a useful test for screening for staphylococci with heteroresistance to vancomycin and those with intermediate resistance to vancomycin, the results also conclusively demonstrated the inducibility of vancomycin resistance by NaCl and β-lactams.

The effect of additional NaCl on the induction of vancomycin heteroresistance is also remarkable. The vancomycin MIC was increased two- to fourfold in the presence of NaCl (table 2, figure 1). This phenomenon is echoed by the fact that

satellitism is also much more pronounced in the presence of 4% NaCl (figure 2). Hence, although additional NaCl is not required for determination of the vancomycin MIC by broth dilution, a higher salt concentration could be essential for the demonstration of heteroresistance, at least in the screening step.

Inducible heteroresistance to vancomycin (table 2) is seen primarily among methicillin-resistant staphylococci (100% in our study), with 83.3% of these 18 strains being CNS. The significance of this finding is uncertain, but it has been well known that some clinical isolates of *S. haemolyticus* and *S. epidermidis* are resistant to vancomycin [19]. We speculate that the emergence of vancomycin resistance might first appear among commensal CNS and perhaps as staphylococci with heteroresistance to vancomycin, which may then spread to *S. aureus,* a hypothesis that is analogous to the postulation that the *mecA* gene accounting for methicillin resistance might have originated from CNS [14, 20]. Assuming that the emergence of vancomycin resistance, like methicillin resistance, is related to antibiotic selection pressure, vancomycin heteroresistance among CNS may herald its appearance in *S. aureus.*

The clinical significance of intermediate vancomycin resistance among *S. aureus* is currently limited to four case reports with at least two treatment failures when glycopeptides were used. Even less is known about its significance in CNS. This is the first case-control study that attempts to answer these questions.

The isolation of staphylococci with heteroresistance to vancomycin was largely from patients in the intensive care unit. This is not unexpected, since the only significant independent risk factors for the isolation of staphylococci with heteroresistance to vancomycin as determined by multivariate analysis were the use of vancomycin within 1 week of bacteremia and admission to the intensive care unit (table 4), and the consumption of vancomycin in our hospital is highest in these wards (data not shown). Although not significant as an independent risk factor, use of β-lactam antibiotics may also be associated with a higher risk of bacteremia due to staphylococci with heteroresistance to vancomycin. Prior use of β-lactams may serve to induce the expression of vancomycin resistance; the sequential use of vancomycin could then exert its effects through selection of vancomycin-resistant subclones in vivo.

It is notable that six of the case-patients received vancomycin and β-lactam antibiotics concomitantly at the time of bacteremia, whereas none in the control group had received this combination. The emergence of staphylococci with heteroresistance to vancomycin, which are invariably methicillin-resistant, could be related to the common practice of empirical β-lactam therapy for sick febrile patients; a lack of response is often followed by addition of a glycopeptide because of the high incidence of superinfection with methicillin-resistant *S. aureus* in our hospital [21]. A prospective study should be done to ascertain the true significance of bacteremia due to vancomycin-heteroresistant staphylococci.

Staphylococci with heteroresistance to vancomycin are likely to be nosocomial pathogens, as their occurrence is associated with nosocomial staphylococcal bacteremia, and all of the isolates in this study were methicillin-resistant. The controls were more likely to have bacteremia due to *S. aureus* ($P < .001$), which is related to the fact that the bacteremic episodes in the controls more frequently represented community-acquired infections and many were due to methicillin-sensitive *S. aureus.* In contrast, 83.3% of the staphylococci with heteroresistance to vancomycin were CNS, which could be related to intravascular catheter–associated sepsis in hospitalized patients. CNS are the most common pathogens causing nosocomial catheter-related sepsis [22]; seven of the cases had the source of bacteremia traced to infected catheters.

The overall mortality from bacteremia due to staphylococci with heteroresistance to vancomycin is higher than that in patients with fully vancomycin-sensitive isolates (44.4% vs. 10%). The difference in mortality is unlikely to be due to the differences in the nature of underlying diseases (table 4) but is probably related to the suboptimal response to the antibiotics used. Nevertheless, direct comparison of the overall mortality rate is unsatisfactory, since the total number of patients is small, and the number of deaths directly attributable to the episode of staphylococcal bacteremia is difficult to ascertain in a retrospective study.

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