JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2008, p. 3124–3126 Vol. 46, No. 9
0095-1137/08/$08.00+0 doi:10.1128/JCM.00592-08

Vancomycin Heteroresistance in Bloodstream Isolates of *Staphylococcus capitis*▽

Danaë D'mello,[1] Andrew J. Daley,[2,4] Muhammad Shihab Rahman,[1] Yue Qu,[1] Suzanne Garland,[2,3,4] Christopher Pearce,[2] and Margaret A. Deighton[1]*

School of Applied Sciences, RMIT University, Plenty Road, Bundoora, P.O. Box 71, Victoria 3083, Australia[1]; Department of Microbiology, Royal Children's Hospital, Flemington Rd., Parkville, Victoria 3052, Australia[2]; Department of Obstetrics and Gynaecology, University of Melbourne, Victoria 3010, Australia[3]; and Department of Microbiology and Infectious Diseases, Royal Women's Hospital, Victoria, 132 Grattan St., Carlton, Victoria 3053, Australia[4]

Received 27 March 2008/Returned for modification 28 May 2008/Accepted 25 June 2008

Nine *Staphylococcus capitis* isolates from blood cultures of newborns were examined for resistance to vancomycin. MICs were within the susceptible range, but population profiling revealed a resistant subpopulation. Only isolates with the largest subpopulation were identified as heteroresistant to vancomycin by Etest. This finding may have therapeutic implications.

Recent reports indicate the possible emergence of *Staphylococcus capitis* as a significant pathogen causing late-onset sepsis in very-low-birth-weight (VLBW) infants (<1,500 g) (6, 16, 18, 20). Reduced susceptibility to vancomycin has been reported in several species of coagulase-negative staphylococci (1, 2, 4, 9, 10, 17); however, there is very little information on the levels of such resistance in *S. capitis*. Reduced susceptibility to vancomycin occurring in methicillin-resistant staphylococci translates into limited treatment options, particularly in newborn infants.

Heteroresistant *S. capitis* isolates may escape detection because MICs of vancomycin of ≤4 μg/ml are generally interpreted as susceptible (5). Agar-based screening tests for detecting heteroresistance in staphylococci are simple to perform (14, 15, 19); however, the sensitivity and specificity of Etest strips are superior (22). Population analysis profiling (PAP) is the most reliable method for detecting heteroresistance, but it is time-consuming and fails to provide results in a clinically relevant time frame (19). Data on the prevalence and level of vancomycin resistance are essential for assessing clinical relevance and treatment options for infants infected with *S. capitis*. This study examines a collection of nine *S. capitis* isolates obtained from blood cultures of VLBW infants in the Neonatal Intensive Care Unit (NICU) at the Royal Women's Hospital, Melbourne, Australia between 1998 and 2002 (3). Pulsed-field gel electrophoresis combined with Southern blot analysis and probing with insertion element IS*256* showed they were closely related yet unique, except for two isolates from the same infant (3). The reference strains used were *S. capitis* ATCC 27840 and *Staphylococcus aureus* Mu3 (ATCC 700698) and Mu50 (ATCC 700699) (11–13).

Isolates were screened for vancomycin heteroresistance on brain heart infusion agar (BHIA) (Oxoid Ltd., Hampshire, England) containing 4 μg/ml of vancomycin (VAN 4) (Sigma-Aldrich Pty. Ltd., Sydney, Australia) (12). MICs were determined by conventional methods and by vancomycin and teicoplanin Etest strips (AB Biodisk, Solna, Sweden) (8), taking care to include small colonies within the clear zone. The PAP profiles were interpreted by calculating area under the concentration-time curve for test and Mu3 (AUC_{test}/AUC_{Mu3}) ratios with the aid of GraphPad Prism 5 software (San Diego, CA) (19, 21). Since the AUC is affected by the size of the initial inoculum, CFUs were standardized to match the initial inoculum of Mu3 for each replicate. All tests were performed on at least three separate occasions.

The various methods differed in their ability to detect resistant subpopulations of *S. capitis*: broth microdilution (CLSI) was the least sensitive method, detecting only one resistant isolate, and the Etest detected three resistant isolates, while VAN 4 screening and PAP analysis detected resistant subpopulations in all isolates (Table 1). Three isolates produced variable results on the VAN 4 screening plates, indicating instability of their heteroresistant phenotype. Visual examination of the PAP graphs showed heterogeneous resistance with strain-dependent differences in the sizes of the resistant subpopulation (Fig. 1). For the three most resistant strains (Mu50 and isolates 15 and 22), there was complete agreement between the results of PAP-AUC analysis, VAN 4 screens, and the Etest. Isolate 6 had a very high PAP-AUC value, produced variable screening results, and was interpreted as nonheteroresistant by the Etest. The discrepant PAP-AUC value could be explained by the unusual shape of the PAP graph, reflecting high colony counts on VAN 2 followed by a sharp drop on VAN 3 plates (Fig. 1). All other isolates and reference strains showed PAP-AUC values close to those of Mu3, but were not heteroresistant according to the Etest. Rankings of isolates according to the size of the resistant subpopulation were generally similar by PAP-AUC analysis and MICs generated by Etests.

These results suggest that a vancomycin-heteroresistant subpopulation is present in all isolates of *S. capitis*. They confirm the unreliability of conventional MICs except for the most resistant isolates and show that the Etest and the VAN 4 screening tests detect only the most resistant isolates but fail to

* Corresponding author. Mailing address: School of Applied Sciences, RMIT University, Plenty Road, Bundoora, PO Box 71, Victoria 3083, Australia. Phone: 61-3-9925 7123. Fax: 61-3-9925 7110. E-mail: m_deighton@rmit.edu.au.

▽ Published ahead of print on 2 July 2008.

TABLE 1. Vancomycin resistance of *S. capitis* isolates

Isolate or strain no.	Result by[a]:								
	PAP-AUC[b]		Vancomycin screen (4 μg/ml)[c]		CLSI standard		Etest		
							MIC (μg/ml)		Interpretation
	Mean (SE) $AUC_{test/Mu3}$ ratio	Interpretation	No. of colonies	Interpretation	MIC μg/ml	Interpretation	Vancomycin	Teicoplanin	
S. capitis 6	1.7 (0.15)	I	7, 86, CG	V	2	S	6	2	S
S. capitis 22	1.7 (0.12)	I	CG	R	4	S	12	16	H
S. capitis 15	1.3 (0.08)	I	CG	R	4	S	12	16	H
S. aureus Mu50	1.6 (0.05)	I	CG	R	8	R	16	12	H
S. capitis 17	1.2 (0.04)	H	4	H	2	S	3	4	S
S. capitis 8a[d]	1.1 (0.03)	H	2	H	1	S	4	3	S
S. capitis 9	1.1 (0.03)	H	0, 2, CG	V	1	S	4	2	S
S. capitis 16	1.1 (0.03)	H	1	H	1	S	3	2	S
S. capitis 8b[d]	1.0 (0.04)	H	12	H	1	S	8	3	S
S. capitis 18	1.0 (0.05)	H	0, 36, CG	V	2	S	4	4	S
S. capitis ATCC 27840	1.0 (0.01)	H	1	H	1	S	2	0.2	S
S. aureus Mu3	1	H	14	H	2		8	16	H

[a] I, intermediate; S, susceptible; H, possibly heteroresistant; R, potentially resistant; V, variable results; CG, confluent growth on at least three of four replicates.
[b] Mean (standard error) AUC determined by PAP. An $AUC_{test/Mu3}$ ratio of ≤0.90 was interpreted as susceptible, a ratio of 0.90 to 1.3 was interpreted as heteroresistant, and a ratio of ≥1.3 was interpreted as intermediate.
[c] Number of colonies on BHIA containing 4 μg/ml vancomycin after 48 h.
[d] Isolates 8a and 8b were collected on different occasions from the same infant.

detect isolates with smaller resistant subpopulations, which could be enriched during vancomycin therapy.

This report is, to the best of our knowledge, only the second to describe a cluster of vancomycin-heteroresistant *S. capitis* strains among VLBW infants in an NICU. Van Der Zwet et al. (18) demonstrated variable proportions of resistant subpopulations in *S. capitis* isolates with closely related or identical genetic profiles. These data suggest that heteroresistant *S. capitis* strains, which are undetectable by standard MIC measurement, could be emerging pathogens in NICUs.

The origin of these nine closely related vancomycin-heteroresistant isolates, present in the NICU for at least 5 years, is enigmatic. It is possible that frequent vancomycin use in the unit led to enrichment of resistant cells present in a common ancestor, resulting in subpopulations of variable size. Although the outcome for *S. capitis* septicemia in VLBW infants is generally good in our unit, it is of concern that further increases in vancomycin resistance could occur. Our observation of a resistant subpopulation in all *S. capitis* strains examined, including ATCC 27840, which was deposited in 1975, suggests that heteroresistance to vancomycin could be an intrinsic property of *S. capitis*. Although more isolates of *S. capitis* need to be examined, given that the relationship between vancomycin heteroresistance and treatment failure is still uncertain (7), it would be wise to consider all isolates as potentially resistant and to monitor clinical responses to vancomycin very carefully,

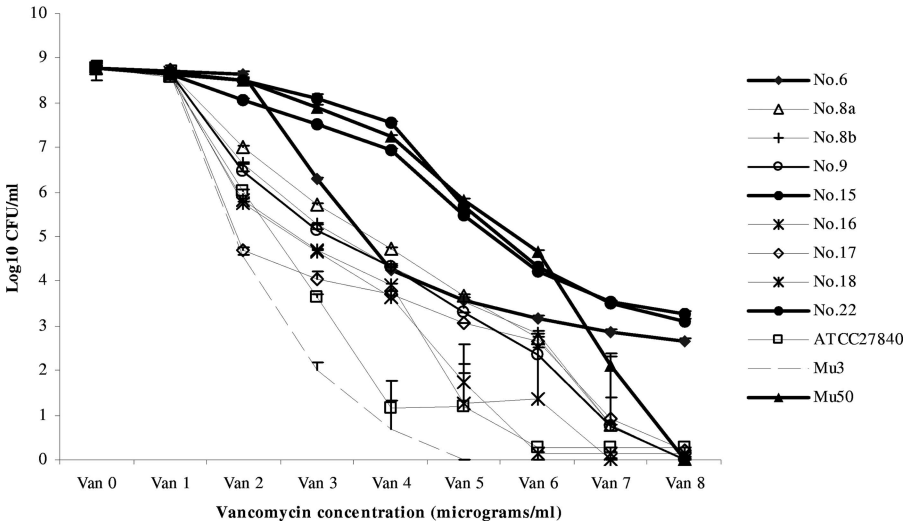

FIG. 1. Population analysis profiling of *S. capitis* strains isolated from blood cultures of infants. Heavy lines, intermediate. The means of three separate investigations and standard errors are presented.

particularly with more deep-seated infections such as osteomyelitis or infections where antibiotic penetration is an issue, such as endocarditis and meningitis. There is an urgent need for more data on the clinical relevance of vancomycin heteroresistance in staphylococci, in particular *S. capitis*, and for the development of reliable, rapid, and inexpensive methods to detect such resistance (7).

This work was supported by internal funding from the School of Applied Sciences, RMIT University, Australia, and the Department of Microbiology and Infectious Diseases, Royal Children's and Royal Women's Hospitals, Australia.

REFERENCES

1. **Biavasco, F., C. Vignaroli, and P. E. Varaldo.** 2000. Glycopeptide resistance in coagulase-negative staphylococci. Eur. J. Clin. Microbiol. Infect. Dis. **19:**403–417.
2. **Biavasco, F., C. Vignaroli, R. Lazzarini, and P. E. Varaldo.** 2000. Glycopeptide susceptibility profiles of *Staphylococcus haemolyticus* bloodstream isolates. Antimicrob. Agents Chemother. **44:**3122–3126.
3. **Bradford, R., R. Abdul Manan, A. J. Daley, C. Pearce, A. Ramalingam, D. D'mello, Y. Mueller, W. Uahwatanasakul, Y. Qu, D. Grando, S. Garland, and M. Deighton.** 2006. Coagulase-negative staphylococci in very low birth weight infants: genetic markers do not distinguish invasive strains from blood culture contaminants. Eur. J. Clin. Microbiol. Infect. Dis. **5:**283–290.
4. **Center, K. J., A. C. Reboli, R. Hubler, G. L. Rodgers, and S. S. Long.** 2003. Decreased vancomycin susceptibility of coagulase-negative staphylococci in a neonatal intensive care unit: evidence of spread of *Staphylococcus warneri*. J. Clin. Microbiol. **41:**4660–4665.
5. **Clinical Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard, 7th ed. Clinical Laboratory Standards Institute, Wayne, PA.
6. **de Silva, G. D. I., M. Kantzanou, A. Justice, R. C. Massey, A. R. Wilkinson, N. P. J. Day, and S. J. Peacock.** 2002. The *ica* operon and biofilm production in coagulase-negative staphylococci associated with carriage and disease in a neonatal intensive care unit. J. Clin. Microbiol. **40:**382–388.
7. **Falagas, M. E., G. C. Makris, G. Dimopoulos, and D. K. Matthaiou.** 2008. Heteroresistance: a concern of increasing clinical significance? Clin. Microbiol. Infect. **14:**101–104.
8. **Fitzgibbon, M. M., A. S. Rossney, and B. O'Connell.** 2007. Investigation of reduced susceptibility to glycopeptides among methicillin-resistant isolates from patients in Ireland and evaluation of agar screening methods for the detection of heterogeneously glycopeptide-intermediate *S. aureus*. J. Clin. Microbiol. **45:**2554–2563.
9. **Froggatt, J. W., J. L. Johnston, D. W. Galetto, and G. L. Archer.** 1989. Antimicrobial resistance in nosocomial isolates of *Staphylococcus haemolyticus*. Antimicrob. Agents Chemother. **33:**460–466.
10. **Garrett, D. O., E. Jochimsen, K. Murfitt, B. Hill, S. McAllister, P. Nelson, R. V. Spera, R. K. Sall, F. C. Tenover, J. Johnston, B. Zimmer, and W. R. Jarvis.** 1999. The emergence of decreased susceptibility to vancomycin in *Staphylococcus epidermidis*. Infect. Control Hosp. Epidemiol. **20:**167–170.
11. **Hanaki, H., K. Kuwahara-Arai, S. Boyle-Vavra, R. S. Daum, H. Labischinski, and K. Hiramatsu.** 1998. Activated cell wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. J. Antimicrob. Chemother. **42:**199–209.
12. **Hiramatsu, K., N. Aritaka, H. Hanaki, S. Kawasaki, Y. Hosoda, S. Hori, Y. Fukuchi, and I. Kobayashi.** 1997. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet **350:**1670–1673.
13. **Hiramatsu, K., H. Hanaki, T. Ino, K. Yabuta, T. Oguri, and F. C. Tenover.** 1997. Methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility. J. Antimicrob. Chemother. **40:**135–146.
14. **Howden, B. P., P. B. Ward, P. D. R. Johnson, P. P. Charles, and M. L. Grayson.** 2005. Low-level vancomycin resistance in *Staphylococcus aureus*—an Australian perspective. Eur. J. Clin. Microbiol. Infect. Dis. **24:**100–108.
15. **Liu, C., and H. F. Chambers.** 2003. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob. Agents Chemother. **47:**3040–3045.
16. **Ng, P. C., V. C. Y. Chow, C. H. Lee, J. M. L. Ling, H. L. Wong, and R. C. Y. Chan.** 2006. Persistent *Staphylococcus capitis* septicaemia in a preterm infant. Pediatr. Infect. Dis. J. **25:**652–654.
17. **Sieradzki, K., P. Villari, and A. Tomasz.** 1998. Decreased susceptibilities to teicoplanin and vancomycin among coagulase-negative methicillin-resistant clinical isolates of staphylococci. Antimicrob. Agents Chemother. **42:**100–110.
18. **Van Der Zwet, W. C., Y. J. Debets-Ossenkopp, E. Reinders, M. Kapi, P. H. M. Savelkoul, R. M. Van Elburg, K. Hiramatsu, and C. M. J. E. Vandenbroucke-Grauls.** 2002. Nosocomial spread of a *Staphylococcus capitis* strain with heteroresistance to vancomycin in a neonatal intensive care unit. J. Clin. Microbiol. **40:**2520–2525.
19. **Walsh, T. R., A. Bolmström, A. Qwärnström, P. Ho, M. Wootton, R. A. Howe, A. P. MacGowan, and D. Diekema.** 2001. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J. Clin. Microbiol. **39:**2439–2444.
20. **Wang, S.-M., C.-C. Liu, H.-W. Tseng, Y.-J. Yang, C.-H. Lin, A.-Y. Huang, and Y.-H. Wu.** 1999. *Staphylococcus capitis* bacteremia of very low birth weight premature infants at neonatal intensive care units: clinical significance and antimicrobial susceptibility. J. Immunol. Infect. **32:**26–32.
21. **Wooton, M., R. A. Howe, R. Hillman, T. R. Walsh, P. M. Bennett, and W. MacGowan.** 2001. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. J. Antimicrob. Chemother. **47:**399–403.
22. **Wooton, M., A. P. MacGowan, T. R. Walsh, and R. A. Howe.** 2007. A multicenter study evaluating the current strategies for isolating *Staphylococcus aureus* strains with reduced susceptibility to glycopeptides. J. Clin. Microbiol. **45:**329–332.