different for the two strains. Both transformants also showed an apparent expansion of the growth inhibitory zone around the cefazidime disc upon addition of the SMA disc.

It has been reported that carbapenem resistance among non-baumannii Acinetobacter spp. is usually due to the production of metallo-β-lactamase.8–10 To our knowledge, this study is the first to report an Acinetobacter spp. positive for blaTMB and to identify a new variant, blaTMB-2. It is also the first report to identify blaTMB in clinical isolates. The low MICs of carbapenem for transformant cells suggests that additional resistance mechanisms, such as the production of other classes of β-lactamase, a reduction in outer membrane protein expressions and an acceleration of efflux pump activities, may be involved in the carbapenem resistance of parental clinical isolates of Acinetobacter spp. Although the same phenomenon was reported in the IMP-type,11 it is notable that one amino acid substitution from serine to proline in TMB-2 has drastically decreased the MICs of meropenem and doripenem. As neither patient had a history of international travel nor any epidemiological link, it is possible that blaTMB-2 had been endemic in Japan but unrecognized because of its reduced ability to hydrolyse carbapenems. The unrecognized spread of blaTMB-2 could be a concern as it can turn to blaTMB-1 by only one nucleotide substitution. Although this report discusses only two cases, it may be important to evaluate the spread of this emerging metallo-β-lactamase gene among non-baumannii Acinetobacter spp.

Acknowledgements
We thank Kumiko Kai, Yumiko Yoshimura and Yoshie Taki for technical assistance and we thank Sunao Matsubayashi and Yuki Koura for providing clinical isolates and information.

Funding
This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H24-Shinkou-Ippan 010).

Transparency declarations
None to declare.

References

J Antimicrob Chemother 2013
doi:10.1093/jac/dkt008
Advance Access publication 1 February 2013

Ability of the VITEK® 2 system to detect group B streptococci with reduced penicillin susceptibility (PRGBS)

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Keywords: group B Streptococcus, GBS, penicillin G

Sir,

Group B Streptococcus (Streptococcus agalactiae, GBS) is the leading cause of neonatal sepsis and meningitis and an important pathogen among elderly people and those suffering from underlying medical disorders.1,2 The highest GBS mortality and morbidity result from invasive infections in neonates.1,2
Approximately 5% of GBS-infected infants die and survivors often suffer from severe neurological sequelae. Intrapartum antibiotic prophylaxis has been recommended by the CDC and is prescribed for pregnant women who have GBS isolated from vaginal specimens. Since the introduction of prophylaxis, the rate of GBS infection during the first post-natal week has decreased. Penicillins are the first-line agents in the prophylaxis and treatment of GBS infections because all clinical GBS isolates have been considered to be uniformly susceptible to β-lactams, including penicillins. However, we identified and characterized several GBS isolates demonstrating reduced penicillin susceptibility (PRGBS) through acquisition of multiple mutations in the penicillin-binding protein 2X (pbp2x) gene, and similar isolates were reported in the USA, Canada, and Japan. After our research was published, EUCAST (http://www.eucast.org/clinical_breakpoints/) defined a clinical penicillin MIC breakpoint for *Streptococcus* groups A, B, C and G, together with the penicillin MIC resistance breakpoint (>0.25 mg/L). The EUCAST breakpoint is higher than the breakpoint for penicillin susceptibility set by the CLSI (0.12 mg/L). Until recently, PRGBS were isolated from respiratory specimens, blood, decubitus ulcers and adult hip-joint fluid, with no report of PRGBS isolated from neonates or vaginal specimens of pregnant women. The isolation rate of PRGBS from various sources is approximately 2.3% in Japan. The MICs of penicillin G for PRGBS (0.25–1 mg/L) are near the breakpoint set by the CLSI (0.12 mg/L). Therefore it is unclear whether automated susceptibility testing machines such as VITEK 2 can detect PRGBS accurately. Because the VITEK 2 system is widely used in clinical laboratories in Japan, we used this system as an example in order to evaluate the ability of automated susceptibility testing machines to detect PRGBS.

The MICs of penicillin G were determined for 28 PRGBS using the agar dilution method as per CLSI recommendations. *Streptococcus pneumoniae* ATCC 49619 was used as a quality control for MIC measurements. It was confirmed that these PRGBS harboured the amino acid substitutions in pbp2x genes, and similar isolates were published. The MICs determined by agar dilution and by VITEK 2 were 0.25–1 mg/L [above the breakpoint set by the CLSI (0.12 mg/L)] for 28 strains ×3 times). The number of strains for which the MICs determined by the VITEK 2 system were ≤0.12 mg/L at least two of three times was 13 (13/28, 46.4%).

In this study, we investigated the ability of the VITEK 2 system to detect PRGBS. It detected only half of the PRGBS in this study. Automated susceptibility testing machines such as VITEK 2 are used in clinical settings worldwide, and these results suggest that many PRGBS may be misclassified as ‘susceptible’ to penicillin G. We recently revealed that PRGBS tends to be resistant to fluoroquinolones and macrolides, in addition to having reduced penicillin susceptibility, indicating that the classification of susceptibility to penicillin G is very important. The worldwide misclassification of PRGBS as ‘susceptible’ to penicillin G is undesirable and hinders attempts to clarify the clinical significance of reduced susceptibility to penicillin G.

The MICs of penicillin G for PRGBS (0.25–1 mg/L) are near the ‘susceptible’ breakpoint (≤0.12 mg/L) set by the CLSI, while the MICs of oxacillin (2–8 mg/L) and ceftizoxime (4–128 mg/L) for PRGBS are higher than those of penicillin-susceptible GBS. However, the VITEK 2 system AST-PS546 cards for *S. agalactiae* do not include MIC determinations of oxacillin or ceftizoxime. We believe that inclusion of these MICs would enable more accurate detection of PRGBS by automated susceptibility testing machines. Moreover, it would be better for these machines to contain systems to alert operators to PRGBS-suspicious isolates when the MICs of penicillin G indicate a range near the susceptibility breakpoint, e.g. at 0.12 mg/L.

Previously we reported that disc diffusion methods using oxacillin, ceftizoxime and ceftibuten were useful for detecting PRGBS. The disc diffusion method for detecting PRGBS does not determine the MIC (mg/L) of penicillin G by VITEK 2.
not require expensive or specialized equipment. Therefore, prior to any improvements in automated susceptibility testing machines, the disc diffusion method for detecting PRGBS will be useful for clinical microbiological laboratories worldwide.

Acknowledgements
We thank Kumiko Kai, Yoshie Taki and Yumiko Yoshimura for technical assistance.

Funding
This study was supported by grants H21-Shinkou-Ippan-008 and H24-Shinkou-Ippan-010, from the Ministry of Health, Labor and Welfare, Japan, and, in part, by a research grant for medical science from the Takeda Science Foundation (2012). The third grant covered the cost of editing by Editage, as mentioned in the Transparency declarations section.

Transparency declarations
The authors have no conflicts of interest to declare. The manuscript was edited by Editage, a language editing company.

References

Ceftaroline in the treatment of concomitant methicillin-resistant and daptomycin-non-susceptible Staphylococcus aureus infective endocarditis and osteomyelitis: case report

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Sir,
A middle-aged male presented to the emergency department with fever and 1 week of sharp right hip pain worsening over the past 2 days. His past medical history included uncontrolled diabetes and chronic active hepatitis C. The patient also had a history of multiple methicillin-resistant Staphylococcus aureus (MRSA) infections, including bacteraemia 1 month prior, for which he was treated with vancomycin (MIC ≤0.5 – 1 mg/L).

Three sets of blood cultures were collected before the patient received a single dose of vancomycin and piperacillin/tazobactam. The blood Gram stain showed Gram-positive cocci. The infectious diseases team was consulted, and given recent vancomycin treatment and recurrent bacteraemia, we recommended high-dose daptomycin (8 mg/kg intravenously every 24 h). Dual therapy with rifampicin was considered, but due to limited in vivo data as well as concomitant hepatic dysfunction, we decided against it.1 Initial blood cultures grew MRSA (3/3 bottles) on hospital day 3, with susceptibility tests showing a daptomycin MIC of 0.38 mg/L (Etest; AB Biodisk, Solna, Sweden) and a vancomycin MIC of 1 mg/L by broth microdilution. Repeat blood cultures on day 4 (2/2 bottles) showed no growth.

On hospital day 2, a transoesophageal echocardiogram (TEE) demonstrated no evidence of vegetations. On day 3, a contrast CT of the hip showed bone and retroperitoneal abscesses along with evidence highly suggestive of osteomyelitis. An MRI was less conclusive about the presence of early osteomyelitis. The surgical team was consulted and recommended that the patient to undergo CT-guided drainage of the retroperitoneal abscess as opposed to any surgical treatment, citing difficult surgical access, a high-risk patient with comorbidities and likelihood of the abnormal synovium in radiographic studies being reactive synovitis as opposed to osteomyelitis and septic arthritis. Drainage proceeded on day 7, though there was continued debate about the diagnosis and need for surgical intervention.