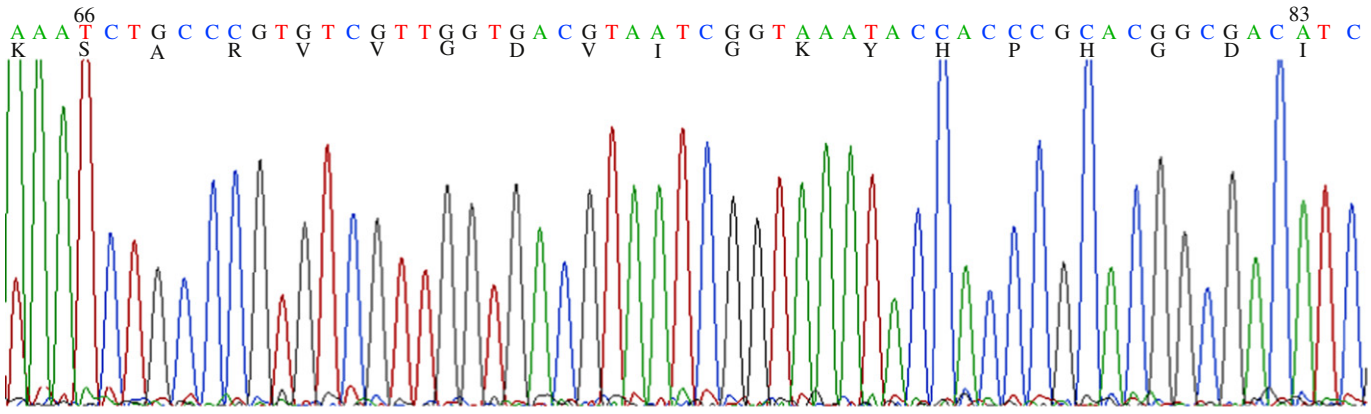

Figure 1. Partial nucleotide sequences of *gyrA* genes of WX3Kpn0010. Mutations were identified with Ser-83→Ile and synonymous substitution, TCA(serine)→TCT(serine), at codon 66.

additive effect on quinolone susceptibility.[7] Though Qnr proteins and Aac(6′)-Ib-cr only induce low level quinolone resistance, they are known to facilitate selection of resistance mutations in the presence of concentrations of quinolone antibiotics.[8]

We describe an isolate of *K. pneumoniae* isolate carrying three plasmid-mediated quinolone-resistant genes (*qnrB*, *qnrS* and *aac (6′)-Ib-cr* variant) together with a novel variant of *gyrA* gene that has not been reported previously. These mechanisms were likely to have contributed individually to the high level ciprofloxacin and levofloxacin resistance.

Conflict of interest statement
None declared.

Funding sources
None.

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Available online 10 July 2010

doi:10.1016/j.jhin.2010.05.007

Vancomycin minimal inhibitory concentration from broth microdilution and Etest in respiratory tract samples of patients with ventilation-associated pneumonia

Madam,

Infections due to meticillin-resistant *Staphylococcus aureus* (MRSA) are a problem in hospitals worldwide.[1] Vancomycin is the treatment of choice for MRSA infections. However, treatment failure is not uncommon, even when the minimum inhibitory concentration (MIC) of vancomycin indicates that the strains are sensitive (MIC $\leq$2.0 μg/mL). Recent studies have demonstrated increased clinical failure of treatment of MRSA infection when isolates have MICs of vancomycin between 1.5 and 2.0 μg/mL.[2,3]

Some authors have used broth microdilution methods or the Etest commercial product for determining vancomycin MICs. So far, the results have shown that vancomycin MICs generated by Etest are higher than those generated by broth microdilution.[4,5]

We evaluated the correlation between vancomycin MICs obtained by broth microdilution and Etest. From June 2009 to February 2010, 18 patients with documented MRSA ventilator-associated pneumonia were included in the study.

Isolates were identified according to standard techniques.[6] Vancomycin MICs were determined by broth microdilution using Clinical and Laboratory Standards Institute (CLSI) methods, and by the Etest method using a 0.5 McFarland inoculum.

The mean age of the patients was 56.7 ± 15 years, and 67% of them were male. Thirty-nine percent of them were immunosuppressed (e.g. human immunodeficiency virus, malignancy, organ transplant). The mean Acute Physiological Assessment and Chronic Health Evaluation (APACHE) II score at diagnosis was 22.8 ± 7.3. All patients were on mechanical ventilation. All patients were treated with vancomycin. The mean length of therapy was 11 ± 8 days, with a mean serum vancomycin level (after the fourth dose) of 24.9 ± 14 μg/mL. The 30 day all-cause mortality was 55.6%.

The mean vancomycin MICs were 0.94 and 0.55, generated by Etest and broth microdilution, respectively. Four (22.2%) of the MICs generated by Etest were 0.5 μg/mL, whereas 88.8% ($N = 16$) of those generated by broth microdilution were 0.5 μg/mL ($P < 0.001$) (Table I).

Two studies have correlated vancomycin MIC values with prognosis in patients with MRSA infection. A retrospective cohort study of 92 patients who received vancomycin to treat MRSA bacteraemia found that MICs ≥1.5 μg/mL were associated with higher mortality. Similarly, Soriano *et al.* evaluated prospectively 414 patients with MRSA bacteraemia, and patients whose isolates had vancomycin MICs >1.0 μg/mL had a higher mortality.[2,3]

In our study, most of the patients had isolates with low vancomycin MIC levels for the MRSA strain (77.7% of them <1.5 μg/mL) by Etest, a finding that differs from those of Soriano *et al.* (26.3% <1.5 μg/mL) and Lodise *et al.* (28.3% <1.5 μg/mL).[2,3]

Comparing MIC values determined by both methods, our results are similar to other studies which found that MICs of vancomycin generated by Etest are one two-fold dilution higher than MICs determined by broth microdilution. Prakash *et al.* analysed 101 strains and found that 89–98% of vancomycin MICs were between 1.5 and 2.0 μg/mL by Etest, but only 3% were 2.0 μg/mL when determined by broth microdilution.[4] Sader *et al.* analysed 1800 strain samples and 96.9% of them exhibited vancomycin MICs ≤1.0 μg/mL by broth microdilution, whereas 58.3% and 32.1% were 1.5 and 2.0 μg/mL, respectively, by Etest.[5] In our results, only 22% of the MRSA strains had vancomycin MICs ≥1.5 μg/mL by Etest, whereas none were ≥1.5 μg/mL by broth microdilution. Swenson *et al.* compared commercial and reference (CLSI broth microdilution) susceptibility testing methods for detecting vancomycin-intermediate *Staphylococcus aureus* (VISA). From a total of 129 samples, Etest had a tendency to categorise susceptible strains as VISA when compared with other methods.[7]

Vancomycin used to be the only treatment option for MRSA pneumonia. A randomised trial compared vancomycin with linezolid in patients with pneumonia.[8] Despite favourable lung pharmacokinetics of linezolid, the mortality rate was similar between groups and the US Food and Drug Administration approved linezolid for treatment of MRSA respiratory infections. Considering our data, and based on the bacteraemia studies by Lodise *et al.* and Soriano *et al.*, for at least 22% of our patients therapy with an alternative agent should be offered (e.g. linezolid, instead of vancomycin) if treatment is based on Etest MIC values.[2,3] However, if broth microdilution MIC values are considered, these patients could have been treated with vancomycin, with optimal trough serum concentrations (15–20 μg/mL).

Swenson *et al.* reported MIC results for 129 samples, but these were not stratified by infection site.[7] The studies by Soriano *et al.* and Lodise *et al.* addressed only patients with MRSA bacteraemia.[2,3] Are these results reproducible in patients with MRSA pneumonia? Can the MICs of vancomycin for strains from patients with MRSA bacteraemia, which are associated with a poorer prognosis, be extrapolated to patients with pneumonia? Should linezolid be the treatment of choice in patients with pneumonia and MICs, obtained by Etest, of >1.0–1.5 μg/mL?

Many points are still unresolved. The number of patients in our study prevented a comparison of mortality rates. Randomised prospective trials with adequate MIC test methodology should address these questions in the near future. In the meantime, we are recommending vancomycin for treatment of MRSA respiratory infections in our setting.

Table I
Comparison of vancomycin minimum inhibitory concentrations (MICs) determined by Etest and broth microdilution

Vancomycin MIC (μg/mL)	No. (%) of isolates with MIC determined by	
	Etest	Broth microdilution
0.5	4 (22.2)	16 (88.8)
0.75	6 (33.3)	–
1.0	4 (22.2)	2 (11.2)
1.5	3 (16.6)	–
2.0	1 (5.5)	–

Conflict of interest statement
None declared.

Funding source
The authors would like to thank the Fundo de Incentivo a - Pesquisa e Eventos (FIPE), Hospital de Clínicas de Porto Alegre, Brazil, for partial support of this study.

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Available online 14 July 2010

doi:10.1016/j.jhin.2010.05.015

Device-associated nosocomial infection in the intensive care units of a tertiary care hospital in northern India

Madam,

Device-associated nosocomial infection is a key factor determining clinical outcome among patients admitted in critical care areas. These infections include catheter-associated urinary tract infection (CAUTI), central line-associated bloodstream infection (CLABSI) and ventilator-associated pneumonia (VAP). The infection control committee of any hospital serves as a major tool for the surveillance of nosocomial infections. Several studies have shown that routine surveillance can reduce the infection by as much as 30%.[1] Hospitals in developed countries generate their infection control surveillance data regularly but there are scanty published data on nosocomial infections available from Indian hospitals. The objective of our study was to ascertain the incidence of device-associated nosocomial infections and the antimicrobial susceptibility patterns of bacterial isolates prevalent in ICUs of our tertiary healthcare centre.

This prospective study was conducted in our 750-bedded hospital, which has two multidisciplinary intensive care units (ICUs) consisting of five and ten beds each. It was carried out from 1 May 2008 to 30 April 2009. Routine surveillance for various nosocomial infections was done by the Department of Microbiology through a specific infection surveillance proforma. The initial samples of every patient admitted to ICU (urine, blood and tracheal aspirate) were sent for bacteriological culture to keep a baseline record. Subsequently, a record was maintained for any of the above infections in the patient during their admission. Antibiotic susceptibility testing of the drugs was carried out following Clinical and Laboratory Standards Institute (CLSI) guidelines using the Kirby–Bauer method.[2] The data were analysed and, based on Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) guidelines, the infection rate was calculated.[3]

The total number of patients admitted over the one-year period was 429. Of these, 105 had 125 episodes of device-associated nosocomial infections. Thus, the overall infection percentage was 24.47% and the infection rate was 29.13%.

All 429 patients had an indwelling urinary catheter and the total number of catheter-days was 5138. UTI occurred in 53 (12.35%) catheterised patients. CAUTI for one year was calculated as 10.2 per 1000 catheter-days. Polymicrobial infection, caused by two organisms, was seen in five cases.

A total of 412 patients had intravascular catheters inserted and the total number of central venous line-days was 3967. Bloodstream infection was detected in 55 (13.34%) patients with central line catheters. The incidence of CLABSI was calculated to be 13.86 per 1000 central line-days.

A total of 400 patients were intubated or had tracheostomies and the total number of ventilator-days was 4125. VAP was diagnosed in 17 (4.25%) ICU patients and the incidence of VAP was 4.12 per 1000 ventilator-days. Polymicrobial infection caused by two organisms was seen in two cases.

The various organisms implicated in CAUTI, CLABSI and VAP were *Pseudomonas aeruginosa* ($N = 34$), *Acinetobacter* spp. ($N = 33$), *Klebsiella pneumoniae* ($N = 21$), *Escherichia coli* ($N = 12$), *Enterococcus* spp. ($N = 19$), *Staphylococcus aureus* ($N = 9$) and Candida spp. ($N = 4$).

A summary of the antibiotic resistance pattern of the isolates implicated in device-associated infections is shown in Table I.

In India, studies done at different centres show considerable variation in infection rates.[4,5] On comparing our rates with those of other centres we found a relatively lower rate of VAP and a higher rate of CLABSI at our centre. This reflects the importance of generating and evaluating individual hospital data for development of proper infection control programmes.

The reason for the higher CLABSI in our ICU setting could be the admission of patients to ICUs at a later stage of the disease usually as a referred case from different hospitals, resource-constrained setting, and the non-availability of hand disinfectants at the patients' bedsides. The relatively lower incidence of CAUTI and VAP may be due to vigilant nursing care, such as adequate cleaning of catheters, emptying of urine bags, nursing the patient in a semi-recumbent position, continuous subglottic suctioning, and strict adherence to ventilator bundle protocol.[6]

As in other studies, *P. aeruginosa* and *Acinetobacter* spp., which are a common cause of nosocomial infections, were also prevalent in our ICUs.[4,7,8]

As predicted, high drug resistance rate and limited drug options for these patients were seen. Gram-negative bacilli were found to be resistant to third generation cephalosporins, β-lactam + β-lactamase drug combination and to carbapenems. Meticillin resistance was observed in 30% of *S. aureus* strains. One disappointing finding was the high (12.5%) prevalence of vancomycin-resistant Enterococci from the samples.

Table I
Antibiotic resistance percentage of various isolates causing healthcare-associated infection

Antibiotic	*Acinetobacter* spp.	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Escherichia coli*	*Enterococcus* spp.	*Staphylococcus aureus*
Cefotaxime	90.3	83.3	96.3	84.6	–	–
Ceftazidime	95.8	94.4	94.1	100	–	–
Piperacillin	64.3	92.9	100	83.3	–	–
Piperacillin + tazobactam	50	77.8	71.4	62.5	–	–
Imipenem	57	76.8	46.7	11.8	–	–
Ciprofloxacin	69.7	61.1	89.5	91.7	80	72.7
Gentamicin	88.9	84.6	91.7	81.8	90	67
Cefoxitin	–	–	–	–	–	30
Vancomycin	–	–	–	–	12.5	0