

B-1

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We have reported that VRSA strain Mu50 has accelerated cell wall synthesis and thickened cell wall. However, the mechanism of vancomycin resistance in other VRSA strains in the world remains unclear. Fifteen VRSA isolates from seven countries were passaged daily in BHI medium without vancomycin supplementation. After 10 to 84 days of passage on non-selective medium, vancomycin-susceptible revertants were obtained from each VRSA isolate as determined by broth dilution MIC test. Most (12/15) of them had hetero-type population curve to vancomycin. Revertants were compared with parent strains for changes in vancomycin-, teicoplanin-, and beta-lactam (imipenem) -susceptibility, cell wall thickness by EM, and correlation between them was analyzed. All vancomycin-susceptible revertants tested had significantly thin cell wall compared to their parents, and there was no significant correlation between the phenotypes of glycopeptide and beta-lactam antibiotic susceptibility. The correlation coefficient of glycopeptide MICs and cell wall thickness for 38 individual isolates was 0.897 for vancomycin, and 0.655 for teicoplanin, respectively. The data indicate that the thickened cell wall is the major contributor to vancomycin resistance but not to teicoplanin resistance; 2) vancomycin resistance phenotype of clinical isolates tend to revert to hetero-type VRSA status by serial passage in drug free medium.

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Evaluation of the Stability of the Glycopeptide Resistance Phenotype in Hong Kong VISA Isolates.

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Three Hong Kong isolates of vancomycin intermediate *Staphylococcus aureus* (VISA) were investigated to assess the stability of the glycopeptide resistance phenotype and evaluate the effects of changes in cell surface. VISA strains were passaged daily for 60 days on both vancomycin supplemented and non-supplemented nutrient agar. MIC for vancomycin was determined at regular intervals. Coagulase tube-test was performed at both room temperature and 37°C using a range of plasma dilutions and the time for clotting recorded. Resistance to lysis by lyostaphin was determined by comparison of survival after incubation for 40 and 60 minutes with lyostaphin. Revertant strains of two of the VISA isolates were produced after 45 days of passaging whilst the MIC of the third strain remained unchanged. The revertants had reduced detection times for clotting of plasma. Those strains continuously passaged in the presence of vancomycin had less coagulase activity and were unable to clot plasma in higher dilution. One revertant showed increased susceptibility to lysis by lyostaphin. Reversion on repeated passage demonstrates the unstable nature of vancomycin resistance in *S.aureus*. Reversion may be due to loss of genetic elements or lack of selection pressure to maintain changes in cell wall structure which facilitate glycopeptide resistance. The isolates demonstrated variation in their ability to revert to a lower MIC as well as production of coagulase and lyostaphin resistance. The reversion time observed was longer than that reported elsewhere. These findings suggest the criteria for stability of vancomycin resistance in *S.aureus* may need to be re-examined.

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Prevalence of Low-level Resistance to Glycopeptides among Staphylococci in a Korean University Hospital

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BACKGROUND Glycopeptides are one of the most frequently prescribed antimicrobial agents in tertiary-care hospitals in Korea, because of a high prevalence of MRSA. The aim of this study was to determine the prevalence of low-level resistance to glycopeptides among staphylococci.

MATERIALS and METHODS A total of 2,279 isolates (1,520 *S. aureus*, 759 coagulase-negative staphylococcus (CNS) isolated between January 1999 and March 2000 were screened by using inoculum of 10⁷ CFU/mL and Mueller-Hinton agars supplemented with 8 µg/mL of teicoplanin (J Microbiol Meth 2000,40: 193-198). For isolates those grown on the screening agar, teicoplanin and vancomycin MICs were determined by agar dilution method according to NCCLS guideline.

RESULTS Of 2,279 isolates, 137 (9.0%) isolates of *S. aureus* and 81(10.7%) CNS grew on the screening agar. The teicoplanin and vancomycin MICs of these strains are summarized as follows: Distribution of teicoplanin and vancomycin MICs in staphylococcus species grown on teicoplanin screening agar

Staphylococcus species	No. of isolates with Teicoplanin MICs (µg/mL)					No. of isolates with Vancomycin MICs(µg/mL) of		
	2	4	8	16	32	1	2	4
(No. of isolates)								
<i>S. aureus</i> (137)	2	15	74	40	6	32	102	3
CNS (81)	2	13	42	22	2	2	73	6
Total (218)	4	28	116	62	8	34	175	8

CONCLUSION The prevalence of low-level resistance to teicoplanin (MIC ≥ 8 µg/mL) of *S. aureus* and CNS was 7.9% and 8.7%, respectively. The prevalence of low-level resistance to vancomycin (MIC ≥ 4 µg/mL) of *S. aureus* and CNS was 0.2% and 0.5%, respectively.

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Complete Structure of Three Types of Staphylococcal Cassette Chromosome mec(SCCmec) Integrated in the Chromosome of MRSA Strains in the World

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Beta-lactam resistance gene *mecA* of *Staphylococcus aureus* is carried by a novel mobile genetic element, designated staphylococcal cassette chromosome *mec* (SCC*mec*) identified in the chromosome of Japanese MRSA strain N315. We now report identification of two additional types of *mecA*-carrying genetic elements found in the MRSA strains NCTC10442 isolated in England and 85/2082 isolated in New Zealand. There were substantial differences in the size and nucleotide sequences between the elements and the SCC*mec*. However, new elements shared chromosomal integration site with the SCC*mec*. Structural analysis of the new elements revealed that they possessed all the salient features of the SCC*mec*: conserved terminal inverted repeats and direct repeats at the integration junction points; conserved genetic organization around *mecA* gene; and the presence of cassette chromosome recombinase (*ccr*) genes responsible for the movements of SCC*mec*. The elements, therefore, were considered to comprise SCC*mec* family of staphylococcal mobile genetic elements together with the previously identified SCC*mec*. Among 38 epidemic MRSA strains isolated in 20 countries, 34 were shown to possess either one of the three typical SCC*mec* elements on the chromosome. Our findings indicated that there are at least three distinct MRSA clones in the world having different types of SCC*mec* in their chromosome.

B-5**Absence of Mutation in the Region(nt. 710-1010) of PBP4 Gene in Clinical Isolates of *Staphylococcus aureus* with Low-level Teicoplanin Resistance**

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BACKGROUND The increasing frequency of clinical isolates of *Staphylococcus aureus* with reduced susceptibility to glycopeptides has evoked the interest in the mechanism of glycopeptide resistance. Recently, two types of abnormalities (a point mutation at nt. 781 and 17-amino acid duplication starting at nt. 915) in *pbp4* gene were reported in a glycopeptide-resistant laboratory mutant. In this study, we investigated these mutations in clinical isolates of *S. aureus* with low-level teicoplanin resistance to see whether they can be used as molecular indicators of glycopeptide resistance.

MATERIALS and METHODS Forty-one strains of methicillin-resistant *S. aureus* - sixteen isolates for which teicoplanin MICs were 16 μ g/mL, nine strains with MICs of 8 μ g/mL, eight isolates with MICs of 4 μ g/mL, eight isolates with teicoplanin MICs were 2 μ g/ml-were used in this study. Teicoplanin MICs were determined by agar dilution susceptibility testing according to NCCLS guideline M7-A5.

Amplification of the region of interest in the *pbp4* gene was performed using a pair of primers (TNM-1. AGCAGTTAGCACCAACAACG; and TNM-2. AGTGC-TAATCCAGCGACAAGG). This resulted in a 571-bp PCR product (nt.680 to 1251, based on the sequences of the *pbp4* gene of *S. aureus* [GenBank accession no.U29454.1]). After successful amplification of the 571-bp PCR products was confirmed, they were subjected to restriction enzyme (*Bcl* I) digestion. For sixteen strains with teicoplanin MICs of 16 μ g/mL, DNA sequences of PCR products were determined with an ABI 377 automated sequencer (Perkin Elmer, Branchburg, NJ, USA).

RESULTS 571-bp PCR products were generated from all 41 isolates indicating absence of 51bp duplication between nt. 680 to 1251. *Bcl* I was selected to identify the sequence variation at nt.781 because the base change (g→t) at nt.781 creates new *Bcl* I recognition site. In addition, *Bcl* I digestion provided an internal control because there is another *Bcl* I site at nt.956. All the PCR products were digested successfully yielding restriction at base 956, and producing two bands (257 bp and 314 bp, respectively). No additional cutting was observed in any of the 41 strains. No mutation was found in the region between nt.710 to 1010 in any of the sixteen-staphylococcal isolates with teicoplanin MICs of 16 μ g/mL sequenced.

CONCLUSION Our results suggest that those changes reported in laboratory-derived high-level glycopeptide-resistant mutants do not exist in clinical isolates of *S. aureus* with low-level resistance to teicoplanin.

B-6**Detection of Multidrug Resistant Patterns and Associated-Genes of Methicillin Resistant *Staphylococcus aureus*(MRSA) Isolated from Clinical Specimens**

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MRSA was obtained from the clinical specimens obtained from the clinical patients at *Pusan National University Hospital, Pusan, Korea*. The sensitivities against various antibiotics were examined by using disc diffusion test and associated genes such as *mecA*, *mecR1*, *mecI* and *femA* were detected by polymerase chain reaction. Among Seventy-nine strains of methicillin resistant *Staphylococcus aureus* (MRSA), 38 strains(48.1 %) were sensitive to streptomycin and 32 strains(40.5 %) to cefoperazone, while one strain(1.3 %) were resistant to vancomycin. In considering the result of this study, 7 strains showed resistance to 9 kinds of different antibiotics, 12 strains were to 8 kinds, 24 strains were to 7, 25 strains are 6, 9 strains were to 5 and 2 strains were to 4 antibiotics. Among seventy-nine strains of MRSA, sixty-seven strains were coagulase positive and twelve were coagulase negative. In the detection of MRSA associated genes by PCR method, *mecA*, *mecR1*, *mecI* and *femA* genes were detected in 30 strains (44.8 %), 28 strains (41.8 %), 23 strains (34.3 %) and 15 strains (22.4 %), respectively. *MecA* type that is without *femA* were found in 21 strains (31.3 %), *femA* type that is without *mecA* were in 6 strains (9.0 %) and *mecA-femA* type were in 9 strains (13.4 %). *MecA* type that is without regulator genes were separated in 4 strains (6.0 %), while *mecA-mecR1-mecI* type that are with regulator genes were separated more to be 17 strains (25.4 %). There was little statistical significance between multidrug resistance and MRSA associated genes. Considering these results, it is to be necessary to include molecular biological studies of related genes in the study of drug resistance.

B-7**Methicillin Resistant *Staphylococcus aureus* : Prevalence, Incidence and Risk Factors Associated with Colonization**

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OBJECTIVE (1) To determine the prevalence of and risk factors for nasal colonisation with Methicillin resistance *Staphylococcus aureus* (MRSA) on admission. (2) To determine the incidence and risk factors for nosocomial acquisition of MRSA.

DESIGN Prospective cohort study between October 1998 and March 1999

SETTING 60 bed male surgical unit in a teaching hospital

PATIENTS 271 Patients admitted for routine surgery

METHODS Nasal swabs were obtained within 36 hours of admission and every other day till discharge. Patients harboring MRSA at the time of admission or after admission were compared with patients who were not colonised. Clinical and epidemiologic risk factors for colonisation were obtained by interview and daily chart review.

RESULT MRSA strains were isolated from 34 patients (12.5%). 20 (7.4%) were colonised on admission and 15(6%) acquired MRSA later. Hospitalisation within the previous year, antibiotic use within the last two months and transfer from another ward of the National Hospital were significantly associated with colonisation with MRSA on admission. A risk factor for nosocomial acquisition of MRSA was the use of antibiotics especially the prophylactic and empiric use of antibiotics. The duration of stay in hospital was significantly longer in patients who acquired MRSA.

CONCLUSION This study helps to elucidate the epidemiology of nasal colonisation with MRSA in a male surgical unit of a teaching hospital. MRSA surveillance and control programs in this ward would be more cost-effective and more likely to succeed if targeted at patients with these risk factors.

B-8**MRSA from the Community: Experience in a University Hospital in HK**Viola Chow, Donald J. Lyon, Augustine FB Cheng
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OBJECTIVE To determine the frequency of MRSA from the community and identify the characteristics of these patients.

METHODS All patients with MRSA isolated from clinical specimens at the Prince of Wales Hospital, Hong Kong from January to March 2000 were identified. Patients with MRSA isolated within 48 hours of admission, who were not transferred from other hospitals nor related to hospital intervention e.g. surgery were included as cases. Medical records were reviewed to determine the demographic data and factors associated with MRSA carriage upon admission.

RESULTS A total of 214 new MRSA cases were identified. 71(33%) have MRSA isolated upon admission.50 (70%) were male. 28 (40%) were infected according to CDC criteria. Commonest infection is lower respiratory tract infection (32%) followed by surgical site infection (25%) and skin and soft tissue infection (18%). 10 (14%) patients died at the same admission. 5 were infected 3 with respiratory tract infection and 2 with bacteremia. All have significant underlying disease. Of the 71 patients, 85% have hospitalization within past 1 year (71% within past 3 months) 27 (38%) have MRSA isolated within past one year. 54 (76%) have antibiotic therapy within past one year and 29 (41%) has catheters at the time of admission. 27 (38%) have surgery within past one year.

CONCLUSION MRSA carriage upon admission is associated with male predominant (70%), previous hospitalization within past 3 months (71%), previous antibiotic use (76%), previous MRSA, catheters and previous surgery.

B-9**MRSA from the Community: Experience in a University Hospital in Hong Kong****V Chow, D J Lyon*, AFB Cheng**

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OBJECTIVE To investigate factors associated with MRSA isolation in clinical specimens from patients on admission to hospital from the community **METHODS** Patients with MRSA isolated from clinical specimens at the Prince of Wales Hospital from January to March 2000 were identified. Patients with MRSA isolated within 48 hours of admission, who were not transferred from other hospitals nor related to hospital intervention, e.g. surgery, were included as cases. Medical records were reviewed to determine the demographic data and factors associated with MRSA carriage upon admission.

RESULTS A total of 214 new MRSA cases were identified. 71(33%) had MRSA isolated upon admission. 50 (70%) were male. 28 (40%) were infected according to CDC criteria. The commonest infection was lower respiratory tract infection (32%) followed by surgical site infection (25%) and skin and soft tissue infection (18%). 10 (14%) patients died during the same admission. 5 were infected, 3 with respiratory tract infection and 2 with bacteraemia. All had significant underlying diseases. Of the 71 patients, 85% had hospitalization within the past 1 year (71% within the past 3 months) and 27 (38%) had MRSA isolated within the past one year. 54 (76%) had antibiotic therapy within the past one year and 29 (41%) had catheters at the time of admission. 27 (38%) had surgery within the past one year.

CONCLUSION MRSA carriage upon admission in Hong Kong is associated with male sex (70%), previous hospitalization < 3months (71%), previous antibiotic use (76%), previous MRSA isolation, catheters, and previous surgery.

B-10**Methicillin *Staphylococcus aureus* (MRSA) IN Cho Ray Hospital****Ha Mai Dung and Vo Thi Chi Mai**

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BACKGROUND The increasing antibiotic resistance of gram-positive cocci has been a growing problem in infection chemotherapy. This situation is the most serious for *Staphylococcus aureus* because of their higher frequency in causing disease than other gram-positive organisms.

OBJECTIVES (1) Define the prevalence rate of methicillin-resistant *Staphylococcus aureus* (MRSA) during the time of 1998 in Cho Ray Hospital, and (2) Evaluate the antibiotic resistance level of these MRSA strains.

MATERIALS and METHOD This is a retrospective and descriptive cross-sectional study about MRSA in 1998 retrieved from Whonet program. *S. aureus* from all kinds of specimens were isolated and identified with conventional method by microbiology lab of Cho Ray Hospital. The susceptibility testing were performed as routine following the standard from NCCLS.

RESULTS MRSA was 46.3% of *S.aureus* isolated in Cho Ray Hospital in 1998. They were isolated from pus (69.8%) and sputum (20.8%). The drug of choice for treatment of severe cases was vancomycin (no resistant strains were detected).

B-11**Methicillin-resistant *S. aureus* in Beijing Children's Hospital, 1993-1998.****Fan JF, Yang YH, et al.**

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OBJECTIVE To explore the antibiotic resistance epidemiological status of *S. aureus* (SA) isolated from Beijing Children's Hospital in the period between 1993-1998.

METHODS Between 1993-1998, 321 SA isolates were collected from children aged 15 days to 15 years. The authors tested 41, 64, 30, 135 and 51 isolates in 1993, 1994, 1995, 1996, and 1998, respectively, for minimum inhibitory concentration (MIC) to 10 kinds of antibiotics with agar dilution method, these antibiotics included penicillin G, oxacillin, erythromycin, tetracycline, chloramphenicol, clindamycin, ciprofloxacin, vancomycin, rifampin and gentamycin.

RESULTS 1) All of 321 isolates were resistant to penicillin G. 2) There was obviously statistical difference in drug resistance rates of SA to 3 kinds of antibiotics ($p<0.05$) between 1993 and 1998. Methicillin resistant *S. aureus* (MRSA) increased significantly from 12.2% in 1993 to 29.4% in 1998. The resistance of SA to clindamycin and ciprofloxacin increased from 51.2%, 2.4% to 72.5% and 17.6% significantly. 3) The resistance of SA to erythromycin, tetracycline, and chloramphenicol during these years were 80%, 70% and 50% or so, respectively. 4) All 321 isolates were exquisitely susceptible to vancomycin, rifampin and gentamycin. 5) Of these SA, 69 (21.5%) isolates were MRSA and 252 (78.5%) were methicillin sensitive *S. aureus* (MSSA). 6) All the 69 MRSA isolates were multiresistant. 96% of the 252 MSSA were multiresistant isolates. There was statistically significant difference in drug resistance rates to 5 kind of antibiotics ($p<0.05$) between MRSA and MSSA. These antibiotics were erythromycin, clindamycin, tetracycline, chloramphenicol and ciprofloxacin. The resistance rates of MRSA to erythromycin, clindamycin, tetracycline, chloramphenicol and ciprofloxacin were 97.1%, 95.7%, 81.2%, 78.3%, and 20.3%, respectively. The resistance rates of MSSA to erythromycin, clindamycin, tetracycline, chloramphenicol and ciprofloxacin were 81%, 53.2%, 67.1%, 43.3%, and 7.5%, respectively.

CONCLUSION Resistance of SA to oxacillin, clindamycin and ciprofloxacin had increased from 1993 to 1998 significantly in the region involved. The degree of resistance and resistance region of MRSA were getting more serious than MSSA. Rational and correct use of antibiotics should be emphasized.

B-12**Pragmatic Approach to the Control of MRSA Infection in a Hong Kong Hospital****T.M.K.So and T.K.Ng**

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BACKGROUND MRSA infection was endemic in the department at a fairly high level, a multidisciplinary team approach involving all health care workers which emphasises the basic hygiene measures of hand washing and standard precautions was adopted as control measures. A pilot observational study on the noncompliance of health care personnel in observing the basic infection control measures revealed (1) improper hand washing technique, (2) no hand washing between contact of different patients or after glove removal, (3) gloves, gowns or masks were not worn during procedures that involve handling the infected patient and/or his secretions, (4) no change of gloves when handling for more than one patient, (5) no cohort measures against infected patient, (6) not aware of the alert for MRSA infected/colonised. The following control measures were enforced to all health care providers: (1) standard precautions with emphasis on hand hygiene, (2) proper hand washing techniques, (3) cohorting MRSA cases, (4) identifying high-risk patient groups which were (I) patients with history of colonization or infection with MRSA within 6 months, (II) inpatients with central venous catheter placement(including haemodialysis catheter) on or after day 3 of insertion, (III) patients with pressure sore of grade 3 or above, (IV) patients with tracheostomy, tracheostomy tube, endotracheal tube, nasopharyngeal tube, with or without ventilator support. Implementation was achieved with registry of the MRSA and the high-risk patients with signage for bedside identification, serial orientation programmes, cohort management of MRSA cases, use of alcoholic hand rub, designated ward liaison nurse for coordination and auditing.

RESULT Over a 12-month period, the compliance rate on hand washing has improved from 90% to 99.5% and the rate of MRSA transmission (MRSA patients/1000 patient days) has dropped from 2.8 to 2.5 in the department.

