Vancomycin-resistant Enterococcus faecium of multi locus sequence type 18 in Malaysia

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SUMMARY

Vancomycin-resistant *Enterococcus faecium* (VREF) in human infections mostly belong to the high-risk, epidemic, clonal complex-17 (CC17) group. Treatment limitation and high conjugation frequency makes it dominant in hospitals worldwide. We investigated positive cultures by Pulse-field gel electrophoresis (PFGE), multi locus sequence typing (MLST). DNA of two strains (A2 and C) appeared to be clonally related by PFGE. Three strains were of ST 18 type (A1, B and C) and strain A2 is of a new ST 596. This ST 18 type strain found in our study is crucial and is believed to be the first in Malaysia.

KEY WORDS:
Vancomycin resistance, Enterococcus faecium, Clonal complex-17

INTRODUCTION

The emergence of vancomycin-resistant *Enterococcus faecium* (VREF) worldwide is worrisome [1]. There are four major global epidemic lineages circulating among humans. However, isolates related to hospital outbreaks reported worldwide are clustered in a genetically closely related group known as clonal complex-17 (CC17) group with Sequence Type (ST) 16, 17, 18, or 20 [2]. These strains present a growing threat to human health as they have developed an increased resistance towards antimicrobial agents. Zubaidah *et al.* [3] reported first confirmed human case of vanA phenotype *Enterococcus faecium* by PCR in Kuala Lumpur Hospital, Malaysia, however there is a need to further characterise these VREF strains by multi locus sequence typing (MLST) from Malaysia due to the lack of information for global comparison. In this study, to the best of our knowledge this is the first report of ST 18 vancomycin-resistant *Enterococcus faecium* in Malaysia.

MATERIALS AND METHODS

Three patients (patient A, B and C) were admitted to Kuala Lumpur Hospital. Patient A, was a 58 year old male admitted to Intensive Unit Care (ICU) in early February 2007 with the diagnosis of septic arthritis and pneumonia. Two tissue samples were collected a month after admission from the right side and left side of the sacral region. Antibiotics that were prescribed over the course of treatment were amikacin (5 days), ceftazidime (4 days), vancomycin (6 doses), meropenem (18 days), fluconazole (10 days), cefepime (4 days) and cloxacillin (7 days). The patient had chronic underlying diseases such as ischaemic heart disease with congestive cardiac failure, hypertension, diabetes mellitus and chronic renal failure. A central venous catheter was also inserted. However, patient succumbed to septic shock despite vigorous treatment.

Patient B, was a 40 year old male, admitted to Urology Ward in early September 2008. A blood sample was collected from the femoral catheter 2 weeks prior admission. The patient was diagnosed with sepsis. Antibiotics that were prescribed over the course of treatment were ceftriaxone (3 days), doxycycline (3 days), vancomycin (3 days), cloxacillin (5 days), meropenem (7 days) and azithromycin (4 days). Other underlying medical conditions were unknown. However, intrajugular catheter, Ryles tube and condom catheter were inserted. Patient had a complete recovery.

Patient C was a 38 year old female, admitted into Intensive Care Unit (ICU) in August 2008 with the diagnosis of community acquired pneumonia with sepsis. A blood sample was collected a month prior admission. Antibiotics that were prescribed over the course of treatment were Sulperazone® (6 days), Tazocin® (6 days), erythromycin (10 days), vancomycin (12 days), cloxacillin (6 days) and ceftriaxone (4 days). The patient had chronic underlying diabetes mellitus and hypertension. Continuous bladder drainage with urinary catheter and Ryles tube were inserted. However, the patient later died of septic shock despite vigorous treatment.

Microbiological investigations

Four strains were processed according to standard laboratory procedures. Antibiotic susceptibility testing was performed by disk diffusion method following standard laboratory procedures. All specimens were identified as *Enterococcus faecium* using Remel RapID Strep Kit (Oxoid, UK). Amplification by PCR showed all strains were positive for vanA gene [4]. Pulse-field gel electrophoresis (PFGE) was conducted with standard protocol [5]. MLST sequences were queried into *Enterococcus faecium* database (http://www.efaecium.mlst.net/) to determine their sequence types. Strains from patient A showed two different MLST types: ST 18 (from the right sore; labelled as A1) and a new ST type, ST 596 (from left sore; labelled as A2) in Figure 1.

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RESULTS

Table I: Antimicrobial Susceptibility Profiles

Patient	Strains	Antimicrobial susceptibility pattern				
		Vancomycin	Teicoplanin	Ampicillin	Gentamicin	Linezolid
A	A1	R	R	R	S	S
A	A2	R	R	R	S	S
B	B	R	R	R	R	S
C	C	R	R	R	R	S

Legend: R; Resistant, S; Sensitive. Only strain C, recorded a vancomycin MIC of >256µgml-1, the MIC for other strains were unavailable.

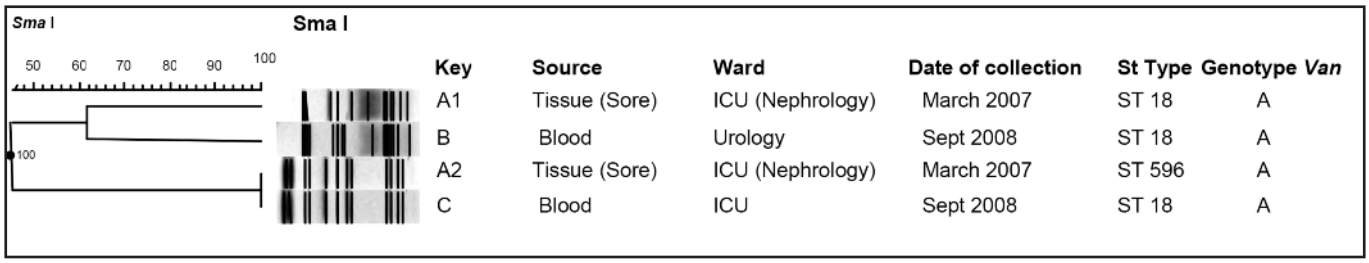

Fig. 1: Dendrogram showing genetic relatedness among sequence types (STs) of VREF isolates cut with SmaI (Bionumerics 6.10, Applied Maths, Saint-Martens-Latem, Belgium). Sources are from clinical isolates. Genotype Van refers to vanA, resistance determinant gene.

DISCUSSION

Samples taken from the target population of this study, from patients with underlying severe urinary and haematology infections was comparable with study done by Zubaidah et al. 3. However, Zubaidah et al. 3 had characterised the VRE strain as VanA by PCR and no further investigation was carried out. MLST genotyping revealed two ST types, ST 18 belonging to high-risk, epidemic, clonal complex-17 (CC17) group and ST 596, a new singleton ST type found in this study. CC17 has been previously described to have associations with ampicillin resistance and harbouring the enterococcal surface protein (esp) pathogenicity island 5. The strains in our study, resembles the characteristics of other strains from other studies around the world 5.

From our PFGE typing investigation, we found that strain A1 and A2 of the same patient have different banding profiles with distinctively different ST types when compared. Though strains A2 and C had similar PFGE patterns (100%), it is not conclusive to claim these strains were clones and a larger sample size needed to verify this claim. In addition, strains A2 and C might be clonally related by PFGE but were of different ST types due to changes in the nucleotides of the housekeeping genes analysed from the ST 18 type. The possibility of having the esp gene in CC17 strains is that the gene contributes to the increased conjugation frequency which could lead to changes in nucleotides of the MLST genes and that can give rise to a new ST type which could not be detected with PFGE. However, these genetic changes demands further investigation.

CONCLUSION

Infection-control measures remain important to prevent the release of VRE of this clonal complex-17 group, a clonal group associated with worldwide nosocomial infection of Enterococcus faecium into the community and vice versa. Little is known about the vancomycin-resistant Enterococcus faecium strains in Malaysian hospitals, and through fourth-generation DNA sequence-based approach in epidemiological typing that the CC17 group was discovered. However, there are limitations to this approach in epidemiological typing due to high costs and technical expertise.

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