BREAK POINT

2016 - ISSUE 17

FROM THE NEWSLETTER EDITOR'S DESK

In this issue, the Newsletter features reports from this year's ASA travel award recipients. Edward Raby, from Fiona Stanley Hospital provides readers with an update on **"Zoonotic transmission of the methicillin-resistant *Staphylococcus* aureus ST612-IV equine strain"** followed by an article from Terence Lee, from Murdoch University entitled "Vancomycin resistant *E. faecium*: Not just a breakpoint issue." On the Horizon is a major highlight of the ASA year, the 16th Asia Pacific Conference on Clinical Microbiology and Infection (APCCMI), to be held in Melbourne, from November 30th to December 3rd, 2016.

Within "In the News" are the infectious risks with one of the newer kinase inhibitors, idelalisib and a note to give us pause of whether we do indeed know how best to wash our hands. As always suggestions towards improving the Newsletter are very welcome.

Sharon Chen
ASA Breakpoint Editor

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IN THE NEWS

IMMUNOCOMPROMISED HOSTS

Risk of *Pneumocystis* pneumonia and cytomegalovirus infections with idelalisib

Idelalisib is a phosphatidylinositol 3-kinase inhibitor used for the treatment of chronic lymphocytic leukemia and non-Hodgkin's lymphoma. Seven clinical trials of idelalisib used in combination with other agents have been halted due to an increase in serious adverse events and fatalities in patients receiving idelalisib [1], with the majority being infections, including sepsis and pneumonia. In particular, an increased number of cases of *Pneumocystis* pneumonia and cytomegalovirus (CMV) infection was observed in the idelalisib groups of three trials [2]. Patients taking idelalisib should receive *Pneumocystis* prophylaxis and be monitored for CMV reactivation and that idelalisib be discontinued in patients with evidence of infection or viremia. Changes to the prescribing information are expected, pending review by the US Food and Drug Administration.

1. Important drug warning (Gilead). Decreased overall survival and increased risk of serious infections in patients receiving ZYDELIG (idelalisib). http://zydelig.com/Content/pdf/Zydelig-Safety-Info-FINAL.pdf.
2. Personal communication, Gilead Medical Information Department.

Technique for hand hygiene with alcohol-based hand disinfectant

Alcohol-containing hand disinfection (AHD) is an effective and practical alternative to soap and water for hand hygiene, but the optimal method of AHD has not been established. The US CDC and Prevention endorses a 3-step method (apply sanitizer and rub both palms together, cover all surfaces, and rub until dry), whereas the World Health Organization endorses a 6-step method (apply sanitizer and specifically rub six different aspects of the hands and fingers).

In a randomized trial comparing these two methods among 120 doctors and nurses at an acute care hospital, those assigned to the 6-step method had a greater reduction in the bacterial count of their hands, but took approximately 8 s longer to complete hand hygiene and had lower compliance (65 versus 100%) [1]. Hospital transmission and infection rates were not measured. We continue to favour the 3-step AHD method because it is practical and the difference in the bacterial count reduction is of uncertain clinical significance.

1. Reilly JS, Price L, Lang S, et al. A Pragmatic Randomized Controlled Trial of 6-Step vs 3-Step Hand Hygiene Technique in Acute Hospital Care in the United Kingdom. Infect Control Hosp Epidemiol 2016; :1.

ZOONOTIC TRANSMISSION OF THE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ST612-IV EQUINE STRAIN

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Zoonotic transmission of methicillin resistant *Staphylococcus aureus* (MRSA) has been predominantly described in relation to livestock associated strains,[1] in particular clonal complex (CC) 398, with elegant studies in the Netherlands demonstrating pig farms as epicentres of MRSA infection.[2] Companion animals have also been implicated however strains in pets more often match the predominant human community strains such as USA100 in the United States and eMRSA15 in the United Kingdom and Europe.[1] Colonisation of horses is less prevalent and MRSA infection in horses remains rare. Here we present two epidemiologically linked cases of MRSA infection with horses as a possible common source.

A 71 yr old lady was being treated for a high grade lymphoma that had transformed from Waldenstrom's macroglobulinaemia. She had had a complete response following 6 cycles of R-CHOP but subsequent ^{18}FDG PET identified early relapse and so she went on to have R-ICE followed by autologous transplant. Less than a week post-transplant she had an episode of febrile neutropaenia and MRSA was cultured from peripheral blood. Blood cultures taken through her central line and subsequent culture of the tip had no growth. Surveillance cultures at 72 hours were negative. Gallium scan showed uptake in the iliacus muscle without a drainable abscess. She initially received piperacillin-tazobactam and vancomycin but developed acute kidney injury and so was switched to daptomycin 500mg q48h for a total of 4 weeks intravenous therapy. Repeat imaging showed reduction in her myositis and again no drainable collection. Her treatment was completed with 2 weeks of oral clindamycin 450 mg tds.

Her MRSA strain was noted to have an unusual antibiogram with resistance to rifampicin, tetracycyline and co-trimoxazole while remaining susceptible to erythromycin, ciprofloxacin and fusidic acid. On further discussion it emerged that her partner had been identified as an MRSA carrier earlier in the year during admission screening. His isolate had a similar antibiogram but in addition was erythromycin and clindamycin resistant. However both were the same rare pulsotype WA20 matched to strain type (ST) 612, a double locus variant of ST8 and within CC8. It also came to light that they lived and worked on a property with 70 horses.

WA20/ST612 is rare in Western Australia with only 9 unique isolates in the MRSA surveillance database that accumulates around 8000 isolates a year (G Coombs, personal communication).

ZOONOTIC TRANSMISSION OF THE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ST612-IV EQUINE STRAIN CONT'D

Four of those isolates were from a clinical source, three from staff and two from patient screening. Five of the isolates had a rural source postcode. Nearly all of these isolates were rifampicin, tetracycline and co-trimoxazole resistant. Clindamycin and ciprofloxacin resistance was uncommon.

In a survey of 771 Australian veterinarians, those in equine practice were found to have the highest MRSA carriage rate at 21.4%, compared to 4.9% of those mainly caring for dogs and cats.[3] ST612 was identified among equine vets but no those in pig or cat/dog practice.[4] Whole genome sequencing of our two isolates and a three veterinarian isolates from this study showed our isolates were closely related within a cluster of ST612 strains (figure). ST612 has also been described in a population of horses in New South Wales.[5] The same unusual antibiogram was observed in both of these studies.[3-5] Worldwide ST612 MRSA remains uncommon in humans except in South Africa where it is the predominant PVL negative community acquired MRSA strain accounting for a third of all MRSA blood culture isolates. [6] In that setting, around 60% of all MRSA isolates are rifampicin susceptible but ST612 is reported to be consistently rifampicin resistant.[7] Widespread use of rifampicin in treatment for tuberculosis has possibly driven this local selection.

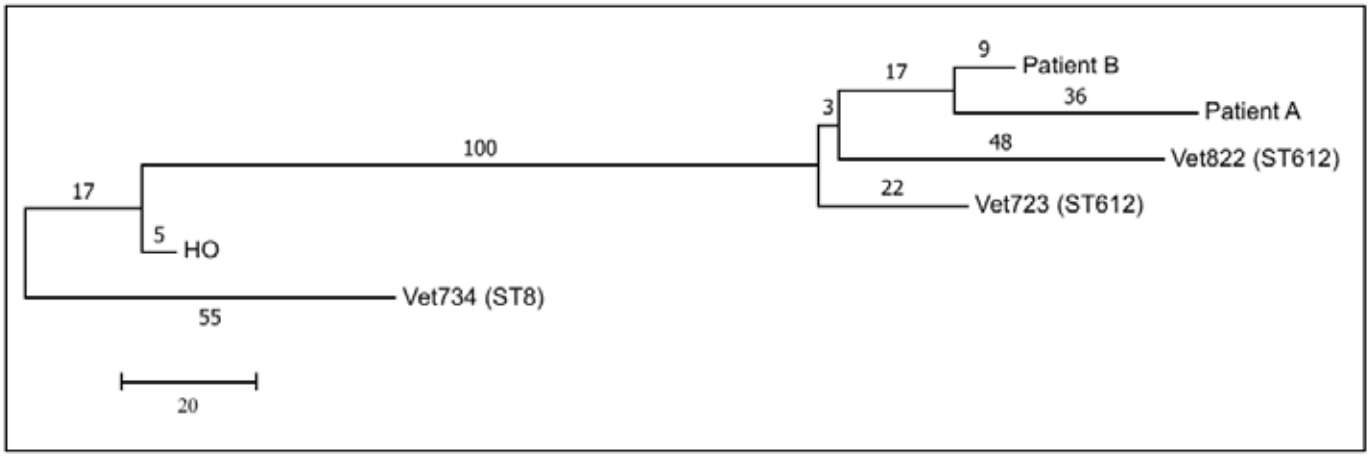

Figure. Maximum likelihood phylogenetic tree based on core and accessory genome SNPs. Patient A and B from this report are seen to cluster with representative strain type (ST) 612 isolates from veterinarian (Vet) screening samples.[4]

Antibiotic pressure probably also explains the predominance of ST612 in horses. Combination therapy with rifampicin and a macrolide is indicated for *Rhodococcus equi* pneumonia also known as rattles.[8] This condition is epidemic on some farms affecting up to 10% of foals with high mortality if treatment is delayed.[9] Equine veterinarians are also five times more likely to regularly prescribe aminoglycosides than those working with other animals.[3] In terms of equine specific virulence factors, an equine pathogenicity island has been described encoding a variant von Willebrand binding protein but this gene was not present in our isolates. It has also been proposed that

ZOONOTIC TRANSMISSION OF THE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ST612-IV EQUINE STRAIN CONT'D

enterotoxin A (*sea*), staphylococcal inhibitor of complement (*scn*) and staphylokinase (*sak*) are redundant in the equine host and so loss of these genes may be an indicator of equine adaptation, however sak and the type D immune evasion cluster including *sea, scn* and enterotoxin O (*seo*) were present in our isolates

The source of infection in our patients remains unclear. Direct transmission is possible but it is interesting to speculate that independent acquisition from their shared horse contact may have occurred and we plan to screen the horses on their property. The rural predominance of the ST612 isolates to date in Western Australia is also intriguing and may represent widespread prevalence of this strain in communities with more frequent horse contact. At this stage however, sporadic importations from South Africa cannot be ruled out. The distribution of this strain elsewhere in Australia has not been defined and it may be overlooked as Aus2/3 if antibiogram alone is used for typing. On a more practical note, this investigation has highlighted the higher prevalence of MRSA in general among those in frequent contact with horses which may be of use when selecting empirical therapy.

1. Weese JS. Methicillin-resistant Staphylococcus aureus in animals. ILAR J. 2010; 51: 233-44.
2. van Cleef BA, Verkade EJ, Wulf MW, Buiting AG, Voss A, Huijsdens XW, et al. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. PLoS ONE. 2010; 5: e9385.
3. Jordan D, Simon J, Fury S, Moss S, Giffard P, Maiwald M, et al. Carriage of methicillin-resistant Staphylococcus aureus by veterinarians in Australia. Aust Vet J. 2011; 89: 152-9.
4. Groves MD, Crouch B, Coombs GW, Jordan D, Pang S, Barton MD, et al. Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus Isolated from Australian Veterinarians. PLoS ONE. 2016; 11: e0146034.
5. Axon JE, Carrick JB, Barton MD, Collins NM, Russell CM, Kiehne J, et al. Methicillin-resistant Staphylococcus aureus in a population of horses in Australia. Aust Vet J. 2011; 89: 221-5.
6. Perovic O, Iyaloo S, Kularatne R, Lowman W, Bosman N, Wadula J, et al. Prevalence and Trends of Staphylococcus aureus Bacteraemia in Hospitalized Patients in South Africa, 2010 to 2012: Laboratory-Based Surveillance Mapping of Antimicrobial Resistance and Molecular Epidemiology. PLoS ONE. 2015; 10: e0145429.
7. Jansen van Rensburg MJ, Whitelaw AC, Elisha BG. Genetic basis of rifampicin resistance in methicillin-resistant Staphylococcus aureus suggests clonal expansion in hospitals in Cape Town, South Africa. BMC Microbiol. 2012; 12: 46.
8. Muscatello G. Rhodococcus equi pneumonia in the foal--part 2: diagnostics, treatment and disease management. Vet J. 2012; 192: 27-33.
9. Muscatello G. Rhodococcus equi pneumonia in the foal--part 1: pathogenesis and epidemiology. Vet J. 2012; 192: 20-6.

VANCOMYCIN RESISTANT *E. FAECIUM*: NOT JUST A BREAKPOINT ISSUE.

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Background

Enterococci are opportunistic pathogens found in the enteric system of humans and animals. Globally *E. faecalis* and *E. faecium* account for almost all reported human enterococcal infections. Over the last two decades, due to the plasticity of its genome, *E. faecium* has amassed a collection of intrinsic and acquired resistance and virulence genes to become a medically important hospital pathogen.

In the 1990s, 90 to 95% of enterococcal infections were due to *E. faecalis* and only 5-10% were due to *E. faecium* (1, 2). In recent years in many countries the prevalence of *E. faecium*, particularly in hospitalised patients, has increased. Furthermore vancomycin resistant *Enterococcus faecium* (*VREfm*) has become a leading cause of nosocomial bacteremia. In Australia, data from the 2013 Australian Group on Antimicrobial Resistance (AGAR) Australian Enterococcus Sepsis Outcome Program (AESOP), showed although *E. faecalis* was the predominant cause of enterococcal sepsis, approximately 40% of cases were due to *E. faecium*, of which approximately 50% were vancomycin resistant (3).

Resistance to vancomycin is conferred by the acquisition of the *van* gene operon which can be categorised into nine *van* types. In Europe and North America vancomycin resistance in *E. faecium* is primarily due to the acquisition of the *vanA* operon (4). However in Australia the majority of *VREfm* harbor the *vanB* operon which can confer variable levels of vancomycin resistance (5). The *vanB* operon can be further divided into three sub-groups, *vanB1*, *vanB2* and *vanB3*, which have so far shown no correlation to the vancomycin minimum inhibitory concentration (MIC).

In our study, we have determined the local epidemiological cutoff (LECOFF) value of wild type *E. faecium* (ie absence of *van* genes) and investigated the distribution of *vanB E. faecium* subtypes on isolates from the 2011 and 2013 Australian Group on Antimicrobial Resistance (AGAR) Australian Enterococcal Sepsis Outcome Programs (AESOP).

VANCOMYCIN RESISTANT *E. FAECIUM*: NOT JUST A BREAKPOINT ISSUE. CONT'D.

Methods

The LECOFF value and *vanB* subtype distribution was determined on 399 wild-type *E. faecium* and 251 *vanB E. faecium* AESOP isolates respectively. Vancomycin minimum inhibitory concentrations (MICs) were performed on all isolates by broth micro-dilution according to the ISO 20776 international standard. The *vanB E. faecium* subtypes were identified by high resolution melt PCR (6).

Results

The vancomycin MIC for the 650 *E. faecium* ranged from 0.125 to >512 mg/L (Figure 1).

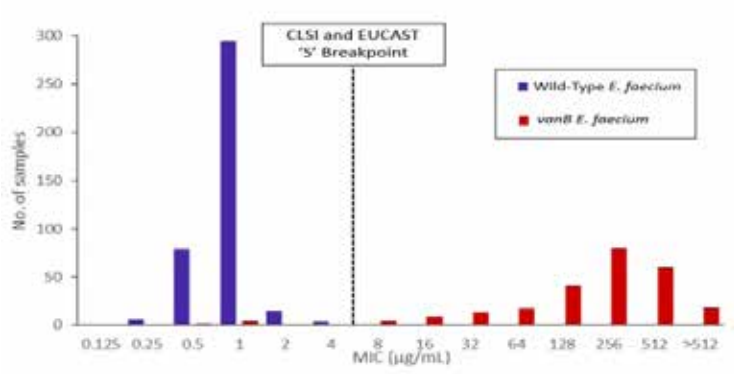

Figure 1: Minimum Inhibitory Concentration Comparison of the wild-type *E. faecium* and *vanB E. faecium*

For the wild-type *E. faecium* the MIC ranged from 0.125 to 4 mg/L, with 73.9% of isolates having an MIC of 1 mg/L. Three isolates had an MIC equal to the CLSI and EUCAST susceptible breakpoint (4mg/L). The LECOFF was 4 mg/L ("eyeball" method,) or 2 mg/L (iterative statistical method).

For the *vanB E. faecium* the vancomycin MIC ranged from 0.25 mg/L to >512 mg/L. Overall 62.9% of *vanB E. faecium* had an MIC ≥256 mg/L. Seven isolates had an MIC <2 mg/L (four isolates 1mg/L; two isolates 0.5 mg/L; and one isolate 0.25 mg/L)

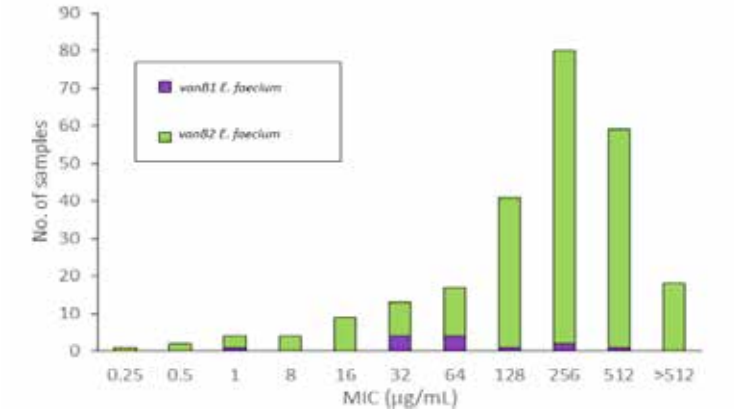

Figure 2: Minimum Inhibitory Concentration Distribution by *vanB* subtype.

VANCOMYCIN RESISTANT *E. FAECIUM*: NOT JUST A BREAKPOINT ISSUE. CONT'D.

Of the 251 *vanB E. faecium* 238 (94.8%) were subtyped as *vanB2*. The *vanB2* MICs ranged from 0.25 mg/L to >512 mg/L with an MIC_{50} and MIC_{90} of 256 mg/L (Figure 2). Thirteen isolates were subtyped as *vanB1*. The *vanB1* MICs ranged from 1 mg/L to 512 mg/L with an MIC_{50} and MIC_{90} of 64 and 256 mg/L respectively. No *vanB3* subtypes were identified.

Of the seven *vanB E. faecium* isolates with an MIC <2 mg/L, six harbored the *vanB2* operon subtype.

Conclusion

We have established for the *E. faecium* tested the vancomycin LECOFF is 2 or 4 mg/L; the same as the ECOFF reported by EUCAST. Although the majority of *vanB E. faecium* had an MIC above the LECOFF, seven isolates had an MIC < 2mg/L, indicating the *vanB* gene in *E. faecium* is not always expressed. In Australia *vanB2* is the dominant *vanB* subtype. The expression of vancomycin resistance is not dependent upon the *vanB* subtype. Until the reasons for the occasional non-expression of *E. faecium* harboring the *vanB* gene are elucidated, we recommend vancomycin susceptible *E. faecium,* particularly when isolated from sterile body sites, be confirmed by molecular testing.

References

1. Witte W, Wirth R, Klare I. Enterococci. Chemotherapy. 1999;45(2):135-45.
2. Moellering RC, Jr. Emergence of *Enterococcus* as a Significant Pathogen. Clin Infect Dis. 1992;14(6):1173-6.
3. Coombs GW, Pearson JC, Daley DA, Le TT, Robinson JO, Gottlieb T, et al. AUSTRALIAN ENTEROCOCCAL SEPSIS OUTCOME PROGRAMME ANNUAL REPORT, 2013. Communicable diseases intelligence quarterly report. 2013;38(4):E320-6.
4. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev. 2000; 13: 686–707.
5. Bell JM, Paton JC, Turnidge J. Emergence of vancomycin-resistant enterococci in Australia: phenotypic and genotypic characteristics of isolates. J Clin Microbiol. 1998; 36: 2187–90.
6. Gurtler V,Grando D, Mayall BC,Wang J, Shahbano GD. A novel method for simultaneous Enterococcus species identification/typing and van genotyping by high resolution melt analysis, J Microbiol Methods; 90:167-181

2016 - 2018 MEETING CALENDAR

Virulence and Resistance in *Staphylococcus aureus*: 2016 State of the Art, ESCMID Postgraduate Education Course
28 June – 1 July, Lyons, France
Website: www.escmid.org

ASM conference on streptococcal genetics
July 31-August 3 2016, Washington DC
Website: www.asm.org

21st International AIDS Conference
17- 20 July, Durban, SA
Website: www.aids2016.org/

16th International Symposium on Staphylcocci and Staphyloccal Infections (ISSSI)
August 30 – September 2 2016 Seoul, South Korea
http://www.isssi2016.org

10th International Transplant Infectious Diseases Conference
Aug 17- 19, Hong Kong, China
Website: www.tts.org/

International Congress for Tropical Medicine and Malaria
Sept 18-22, Brisbane, Australia
Website: http://tropicalmedicine2016.com/

Infection Prevention 2016
Sept 26-28, Harrogate, England
Website: www.ips.uk.net

ID week 2016
Oct 26-30, New Orleans, LA
Website: www.idsociety.org

MED 2016: International Meeting on Emerging Diseases and Surveillance
4-7 November, Vienna, Austria
Website: http://imed.isid.org

16th Asia Pacific Conference on Clinical Microbiology and Infection (APCCMI)
30 Nov- 3 Dec, Melbourne, Australia
Website: http://www.asainc.net.au

2017

ASA Annual Meeting, in conjunction with the StaphPath Meeting
23-25 February, Adelaide, Australia
Website: www.antimicrobials2017.com

British Society for Microbiology Annual Meeting
3-6 April, Edinburgh, Scotland
Website: www.microbiologysociety.org

27th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2017)
22-25 April 2017, Vienna, Austria
Website: http://escmid.org/dates_events/

ASM Microbe 2017
1-6 June 2016, New Orleans, LA
Website: www.asm.org/microbe2017

ID week 2016
Oct 4-8, San Diego, CA
Website: www.idsociety.org

2018

ASA Annual Meeting
22-24 February, 2018 Sydney, Australia
Website: www.antimicrobials2017.com

28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2018)
21-24 April 2018, Madrid, Spain
Website: http://escmid.org/dates_events/

ASM Microbe 2018
7-11 June 2016, Atlanta, GA
Website: www.asm.org/microbe2018