

CLINICAL ALERT

Emergence of vancomycin-resistant *Enterococcus* at a tertiary paediatric hospital in South Africa

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Background. During 2013, the haematology/oncology unit at a tertiary level paediatric hospital in South Africa experienced the emergence of infection with vancomycin-resistant *Enterococcus* (VRE).

Objective. To describe the clinical and molecular aspects of the cases identified.

Methods. VRE isolates identified from blood culture specimens processed at the National Health Laboratory Service were screened for the presence of the vancomycin resistance genes *vanA*, B and C1, 2 and 3. Further characterisation of these isolates was carried out using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Clinical records of infected patients were reviewed to identify possible risk factors, while surveillance with rectal swabs was performed to identify VRE-colonised patients.

Results. Four patients with haematological malignancies were identified with VRE bloodstream infections. Patients were immunocompromised at the time of the bloodstream infection (BSI), with receipt of vancomycin prior to VRE-BSI, and infections were treated with linezolid. Colonisation with VRE was found in 8 of 55 patients screened. Infected and colonised patients were isolated in the unit during their admission and strict contact precaution infection control practices were instituted. The *vanA* gene was identified in all of the isolates but one. PFGE and MLST results showed a degree of genetic relatedness between certain isolates obtained from rectal swab and blood culture samples, suggesting possible patient-to-patient transmission or persistence of the isolates in the unit.

Conclusion. Strict infection control practices are necessary to prevent infection and transmission of resistant organisms among vulnerable patients.

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Multidrug-resistant organisms are an increasing threat to health-care worldwide. Treatment options for these so-called 'superbugs' are limited, with very few new antimicrobials currently in development.

One such organism is vancomycin-resistant *Enterococcus* (VRE). This organism has been detected worldwide in hospitalised adult and paediatric patients since the first description in 1986.^[1] Infection and/or colonisation with VRE has been described in patients who are potentially immunosuppressed, especially those with malignancies, solid organ transplant recipients, those with chronic renal disease requiring haemodialysis, neonates and those requiring long-term ventilation.^[2,3]

Resistance to vancomycin occurs in *E. faecium* and *E. faecalis* species primarily owing to the *vanA* and *vanB* resistance genes. The first clinical reports of VRE in South African (SA) adults appeared in 1997 and there is currently a paucity of SA paediatric studies describing the incidence of VRE or outbreaks of this organism.^[4]

We report on the first identification of VRE infections in the paediatric oncology ward at a tertiary-level paediatric hospital in Cape Town, SA, over two time periods in 2013.

Methods

Identification

The first two VRE organisms causing bloodstream infections (VRE-BSI) were isolated from blood culture specimens of two patients, 2 days apart, in February 2013. Seven and 9 months later, in September and November 2013, respectively, VRE was once again isolated from blood culture specimens in an additional two patients. Following the two time periods, patient hospital records were accessed to identify possible risk factors associated with acquiring VRE-BSI in this setting. Patient records were anonymised and de-identified prior to analysis. Routine screening is not carried out for drug-resistant organisms on admission to the hospital.

Setting

The haematology/oncology unit at Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, SA, is a 17-bed unit with a high-care unit and isolation cubicles. There is a daily outpatient service and a day ward in the area adjoining the main ward. It serves as a referral centre for children with haematological and solid organ malignancies from other local or distant hospitals in the country. Children are admitted from home or long-term

care facilities. The unit has approximately 2 000 admissions, with an additional 5 500 outpatient visits, per year

Surveillance

Following identification of the first two VRE infections, collaboration between the paediatric oncologists, microbiologists, paediatric infectious diseases physicians, infection control nursing staff and ward nursing staff was instituted to limit further spread by improving infection prevention and control measures. Active surveillance for rectal colonisation with VRE of patients in the ward and discharged patients who had prior contact with the index cases was carried out by means of culture and sensitivity testing using rectal swabs or stool samples. If rectal swabs were negative for VRE at initial screening, a repeat was taken at 1 - 2-weekly intervals until three consecutive negative swabs were documented.^[5]

Infection control

Infected patients were isolated in single cubicles until discharged. Contact precautions during patient interaction were instituted for patients, family and staff members. Colonised patients were cohorted until discharged. Medical records were marked to ensure isolation, and re-screening by rectal swab occurred during subsequent admissions. Staff and parents were re-educated regarding correct handwashing technique, use of alcohol hand sanitiser and contact precautions.

Microbiological methods

All microbiological and molecular analyses were conducted by the National Health Laboratory Service at Groote Schuur Hospital and the Division of Medical Microbiology, University of Cape Town (UCT), SA. Surveillance specimens were plated directly onto selective media (colistin nalidixic blood agar impregnated with a 30 µg vancomycin disc) and incubated for up to 48 hours. Identification and susceptibility testing of colonies growing within the inhibition zone close to the vancomycin disc were performed using the Vitek 2 GP and AST-P603 cards respectively, with interpretation according to contemporary Clinical Laboratory Standards Institute criteria.^[6] Vancomycin minimum inhibitory concentrations (MICs) were determined using the Etest method.^[6] MICs ≥32 µg/mL were considered resistant.

Isolates were characterised genotypically with the Hain Genotype *Enterococcus* line probe assay according to the manufacturer's instructions, or in-house polymerase chain reaction (PCR) assay as described below.

In-house PCR assay for genotyping enterococci

All vancomycin-resistant isolates were screened for the presence of the vancomycin resistance genes *vanA*, B and C1, 2, 3 with DNA purified using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, USA) according to the manufacturer's instructions.^[7] PCR reactions (25 µL) were carried out using GoTaq Flexi (Promega, USA) with an annealing temperature of 60°C, for 35 cycles, in a GeneAmp 9700 thermocycler. PCR amplicons were visualised by agarose gel electrophoresis and ethidium bromide staining. Amplicons were confirmed with DNA sequence analysis (Inqaba Biotech, SA) and BLASTN (<http://blast.ncbi.nlm.nih.gov/Blast>) comparisons.^[8]

Pulsed-field gel electrophoresis

Isolates of interest were analysed using pulsed-field gel electrophoresis (PFGE), as described by the Centers for Disease Control and Prevention, with the following changes: cultures were grown on BHI medium, at 37°C, aerobically overnight, and cell densities were standardised to an OD_{610nm} ≈ 1.0, using a spectrophotometer (BioDrop, UK).^[9] Cells were embedded in a 1.0% SeaKem Gold agarose gel (Lonza, Switzerland) and were digested for 2 hours at 25°C using *SmaI* (Thermo Scientific, USA) restriction endonuclease and *ApaI* as a secondary enzyme (2 hours at 37°C). Digested plugs were electrophoresed through a 1.0% SeaKem Gold agarose gel (Lonza, Switzerland) in 0.5% TBE buffer (14°C), using the Gene Navigator (Amersham Biosciences, UK) at 200 V, with a ramped switching time of 3.5 - 23.5 seconds over 18 hours. The resultant gel was visualised using ethidium bromide staining, and the gel image analysed and dendrogram generated using Gel Compare II (BioNumerics v.6.6, Applied-Maths, USA), with an optimisation of 1.0 and a band tolerance of 1.5. The dendrogram was constructed using the pairwise average from the *SmaI* and *ApaI* results, with the UPGMA (unweighted pair group method with arithmetical mean) method and branch quality was calculated using the cophenetic correlation. *E. faecalis* ATCC 51299 was used as a control and for normalisation.

Multilocus sequence typing

Cellular DNA was extracted from selected isolates and PCR was carried out as described above using the conditions described by Homan *et al.*^[10] Amplicons were sequenced bidirectionally (Inqaba

Table 1. Summary of patients with VRE-BSI

Patient	Rectal screening swab	Underlying diagnosis	Duration of hospitalisation before BSI (days)	Immunosuppressive therapy	Mucositis	Duration of neutropenia* before BSI (days)	Central venous catheter	Vancomycin use before specimen (days)	Vancomycin trough levels (µg/mL)	Linezolid duration (days)	Outcome
1	Positive	Acute promyelocytic leukaemia	67	Maintenance chemotherapy	Yes	11	Yes	6	4.2	10	Alive and discharged from the unit
2	Positive	Aplastic anaemia	77	Cyclosporin	No	77	Yes	13	29.6	14	Died of septic shock
3	Not done	Burkitt's lymphoma	23	Induction chemotherapy	Yes	9	Yes	3	9.8	3	Died of septic shock
4	Positive	Acute myeloid leukaemia	63	Maintenance chemotherapy	No	14	Yes	8	13.7	11	Alive and received an HSCT

HSCT = haemopoietic stem cell transplant.
*Neutropenia: absolute neutrophil count 50.5×10^9 cells/L.

Biotech, SA) and the consensus sequences uploaded and typed at using an *E. faecium* multilocus sequence typing (MLST) database.^[11]

Ethical considerations

Ethics approval to perform this study was obtained from the Human Research Ethics Committee, UCT (HREC Ref. No. 022/2015).

Results

Description of the infection

The ward had 100% occupancy during the months of VRE isolation. All four BSI patients were long-term residents in the unit at the time of the infections and were therefore classified as hospital-acquired infections. Patients 2 and 4 were transferred from a referral hospital outside Cape Town, where VRE had previously been isolated. Neither of these patients was screened prior to their transfer to RCWMCH. The clinical features and underlying risk factors at the time of BSI were similar in all four identified patients (Table 1). Three of the infected patients cultured vancomycin-sensitive *E. faecium* (VSE) isolates from blood in the week preceding the VRE-BSI. All four were treated with vancomycin in the week prior to the the VRE-BSI, and three had suboptimal vancomycin trough drug levels ($\leq 15 \mu\text{g/mL}$). Patient 2 had renal impairment which resulted in the increased trough levels of vancomycin. Central venous catheters were removed from all infected patients after detection of the VRE-BSI. Infected patients were treated with intravenous linezolid. Patients 2 and 3 had protracted periods of illness prior to the BSI and demised from a new Gram-negative BSI and typhilitis following the VRE-BSI, respectively.

Surveillance

Two of 29 (6.9%) patients who were residing in the ward or recently discharged were screened (as identified above) by rectal swab for VRE during February and March 2013 and a further 6 of 26 (23.1%) patients screened between September and October 2013 were positive for VRE. Patient 4 screened positive on rectal swab and

subsequently progressed to develop a VRE-BSI a week later. Although the overall number of patients colonised with VRE was 14.5% (8/55), surveillance swabbing was not routinely performed between March and September, and the true prevalence was therefore not established. There were, however, no cases of clinical disease due to VRE documented between March and September 2013. Environmental screening for VRE was not conducted in the unit.

Microbiology

All but one isolate obtained (surveillance and infection) was identified as *E. faecium*, using the Vitek 2 system. The additional isolate obtained on rectal swab was identified as *E. faecalis* based on vancomycin sensitivity testing. Vancomycin MICs on Etest were $\geq 256 \mu\text{g/mL}$ for the four BSI isolates, confirming high-level resistance of *E. faecium* to vancomycin.^[8]

Molecular testing

PCR analysis detected the *vanA* gene in all of the VRE isolates obtained from infected patients and all but one of the VRE screening isolates. This isolate tested positive for the *vanB* gene. PFGE analysis of these isolates showed that there was genetic diversity among the isolates, which was not indicative of an outbreak. There was, however, a high level of genetic relatedness ($>85\%$ confidence interval) between some of the strains tested (Fig. 1). The *E. faecalis* isolate obtained during the February - March surveillance screening did not undergo any further molecular typing, even though the *vanA* gene was detected on PCR.

Patients 1 and 2, who were considered to be the index cases of a suspected outbreak, had isolates which shared 92.5% homology, indicating a strong possibility of patient-to-patient transmission of the same clone. This result was unusual since the patient 2 isolate in question (No. 13) was obtained from a rectal swab and the isolate obtained from the blood culture of Patient 2 (No. 3) did not show strong homology with each other.

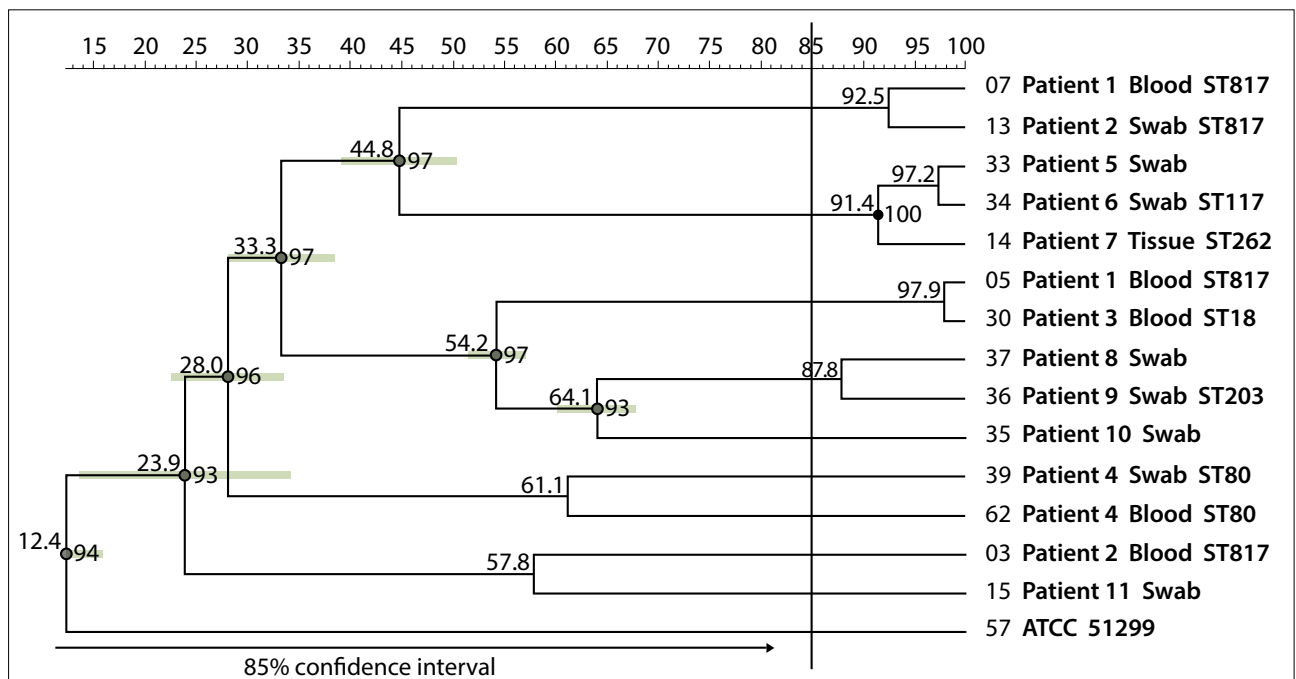


Fig. 1. Dendrogram of isolates obtained during investigation. Shaded bars indicate branch error flags and unshaded values indicate the levels of relatedness. Patients 1 - 4 represent the patients with VRE-BSIs. Patients 5, 6 and 8 - 11 indicate the patients colonised with VRE (patient 11 in February and March, and patients 5, 6, 8, 9 and 10 in September and October; patient 7 was not a haematology/oncology patient). The specimen types and MLST sequence types are indicated in the last two columns, respectively.

The second isolate from patient 1 (No. 5), obtained from a blood culture, shared 97.2% homology with the blood culture isolate from patient 3 (No. 30). Isolate No. 30 was faintly positive for the *vanA* gene on PCR and susceptible to vancomycin. Previous isolates from this patient produced strong positive PCR results and high levels of vancomycin resistance. None of the resistant isolates from patient 3 showed significant homology to the sensitive isolate or to any of the other strains tested (data not shown). The two isolates obtained from blood in patient 1 (Nos 5 and 7), however, shared very little genetic homology. These data indicated the possibility of a rapid rate of strain variation, even within the same patient, in this setting. The two isolates from patient 4 (Nos 39 and 62) shared only 61.1% homology with each other and very low homology with any of the other strains tested.

There were five screening strains, in two clusters, that shared high genetic homology with each other. Cluster 1 (91.4% homology) comprised patients 5, 6 and 7 and cluster 2 (87.8% homology) comprised patients 8 and 9. Patient 7 (No. 14) in cluster 1 was PCR-positive for the *vanB* gene, but still shared high homology with the other two isolates, which were both *vanA*-positive. This patient had no prior contact with the haematology/oncology unit. The isolate was obtained from autopsy of the lung tissue and was probably a contaminant and not a comorbidity. Cluster 2 comprised two isolates that were obtained from different patients on the same day. These results indicated the possibility of spread within the hospital.

MLST analysis revealed that the four isolates from patients 1 and 2 (Nos 3, 5, 7 and 13) all shared the same sequence type (ST817). Even though these isolates do not share significant homology in the dendrogram, it strengthens the assumption that these two patients probably had patient-to-patient transmission of the same clone that may have changed over time. The two isolates from patient 4 (Nos 39 and 62) that shared 61.1% homology also shared the same sequence type (ST80). They are single-locus variants of ST817, with a single polymorphism in the *adk* gene (189G>A).

Infection control

A multidisciplinary approach was instituted to prevent further spread of the organism within the oncology unit. Infection control staff assisted with ensuring that infected patients were isolated and all ward staff members were educated about contact precautions. Colonised patients were cohorted in the multi-bed cubicles for the duration of their hospital stay. Discharged patients were identified to determine who required screening on their next visit.

The ward was closed off to all new admissions until all colonised or infected patients were discharged. Once all colonised or infected patients were discharged, the ward was comprehensively cleaned.

Following the first two cases of VRE-BSI, antibiotic stewardship ward rounds were instituted in the oncology unit, to improve antibiotic management including optimisation of vancomycin dosing. All patients who required transfer from other hospitals to the oncology unit at RCWMCH, where VRE had previously been documented, were screened for VRE by rectal swab prior to transfer.

Discussion

This is the first documented report of VRE infection in children in SA. A prevalence study in SA found 10.9% (20/184) of screening rectal swabs in at-risk patients to be positive for enterococcal isolates.^[12] This study also showed clonal spread of the *vanA* and *vanB* genotypes in different hospitals, as well as persistence of the *vanA* carrying strain over time. The *vanA* genotype has been commonly isolated in previous outbreak settings.^[2,3]

Even though the PFGE results indicated overall genetic diversity between the isolates analysed, there was a degree of genetic relatedness

between specific blood and rectal swab samples on both PFGE and MLST analysis. These results may suggest possible patient-to-patient transfer of VRE or persistence of the organism in the haematology/oncology unit between the two time periods described. Patient 4 was infected by a molecularly distinct, unrelated isolate, possibly before transfer to RCWMCH at a referral hospital where VRE had previously been isolated. The identified sequence type (ST80) along with ST203 (No. 36) and ST18 (No. 30) have previously been assigned to clonal complexes (CC) CC117, CC78, and CC17, respectively, which are known to form part of the CC17 meroclone. The CC17 meroclone has been described as highly diverse with documented worldwide spread.^[13] These data again highlight the high levels of strain variation observed in this setting.

The purpose of screening is to identify carriers during outbreaks, and in non-outbreak settings to prevent transmission, especially to vulnerable patient groups.^[14] Active screening outside outbreak settings can be a costly exercise and especially difficult in resource-constrained health systems. Screening in children has revealed low rates of colonisation. Active screening of paediatric patients found that 3.4% (41/1 211) were colonised with VRE, of whom 39/41 (95.1%) had an underlying chronic disease.^[15]

Risk factors for acquiring a BSI or colonisation with this highly resistant organism have been investigated. There are few paediatric studies regarding the risk factors for acquiring VRE. Acquisition of VRE has been associated with previous treatment with broad-spectrum antimicrobials, especially third-generation cephalosporins, and the carbapenems, as well as immunosuppression with antineoplastic chemotherapy, haematology/oncology and renal patients, increased length of stay in hospital, and a younger age in children.^[13] Progression from VRE colonisation to VRE-BSI in patients was associated with admission from a long-term care facility, infection of an additional body site other than blood and receipt of vancomycin.^[16] In contrast, Pentima *et al.*^[17] found that VRE-BSI was independent of patient vancomycin exposure. Risk factors in our patient cohort are similar to those described. All the patients with VRE-BSI had an underlying haematological condition requiring immunosuppressive therapy for treatment and received vancomycin for treating presumed Gram-positive infections. Central venous catheters were present in all four infected patients and, although not found to be a risk factor in the studies mentioned, could represent a possible clinical source for BSI. The colonised patients, although not clinically ill at the time, also had similar risk factors: all were exposed to vancomycin at some period during their illness and had underlying immunosuppression in the period preceding the screening. As mentioned, only one of the colonised patients progressed to developing bacteraemia with VRE.

Treatment for this resistant organism is limited to the use of linezolid and daptomycin. In SA, daptomycin is difficult to access in the public health system. Linezolid, an oxazolidinone antibacterial agent, has proven to be effective in the treatment of the glycopeptide-resistant *Enterococcus*, as well as methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* in children, and was used to treat the infected patients in this study.^[18]

Study limitations

The limitations of this study include lack of a case-control group to conclusively identify risk factors associated with VRE acquisition in our patients, and an insufficient sample size to ascertain whether an outbreak had occurred and to determine the definitive phylogenetic relationships of the VRE strains present in this setting. Owing to financial constraints, no active surveillance was possible after October 2013, so whether or not further transmission occurred and

the duration of colonisation with VRE were not established. However, no further VRE cases were reported in the oncology unit or the hospital after November 2013.

Conclusion

In conclusion, effective infection control practices are important to limit or prevent transmission of multidrug-resistant pathogens among vulnerable hospitalised populations. Furthermore, the emergence of glycopeptide-resistant enterococci has emphasised the importance of antibiotic stewardship in the haematology/oncology unit and the institution overall.

Conflicts/disclosures. Parts of this work have been previously presented as a poster at the 16th International Congress on Infectious Diseases (ICID) in 2014 and published in the conference proceedings in the *International Journal of Infectious Diseases* (2014), as well as a poster presentation at the 9th World Congress of the World Society for Pediatric Infectious Diseases (WSPID) in 2015.

Authors' contributions. HL and CM participated in the drafting of the manuscript. BE conceived the study and helped in the drafting of the manuscript. CM and DR carried out the molecular typing of all the isolates. All authors read and approved the final manuscript.

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