Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Français | Contact Us | Help | Search | Canada Site
Home | Centres & Labs | Publications | Guidelines | A-Z Index
Child Health | Adult Health | Seniors Health | Surveillance | Health Canada

Public Health Agency of Canada (PHAC)

Canada Communicable Disease Report CCDR

Volume 27-12
15 June 2001

[Table of Contents]

IDENTIFICATION OF THE FIRST VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECALIS* HARBOURING *vanE* IN CANADA

Introduction

The first isolate of vancomycin-resistant *Enterococcus* (VRE) in Canada was reported from Edmonton in 1993[1] and the first published outbreak of VRE in Canada occurred in Toronto, Ontario in 1995[2]. In Canada, surveillance for VRE is passively reported to Health Canada through the Canadian Nosocomial Infections Surveillance Program (CNISP) at the National Microbiology Laboratory (NML). The VRE-Passive Surveillance Network of CNISP found VRE in 113 health care facilities in 10 provinces, for a total of 1,315 cases of VRE (95% colonized, 5% infected)[3]. Recently, several provinces have made VRE a reportable condition to the Provincial Medical Officer of Health.

Glycopeptide-resistant enterococci are classified genotypically into five main groups. The *vanA*-type strains display high level resistance to the glycopeptides vancomycin and teicoplanin[4] whereas the *vanB*-type strains show variable levels of resistance to vancomycin[5]. Intrinsic low-level resistance (8 mg/L to 16 mg/L) to vancomycin, but not teicoplanin, are found in *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens* harbouring the chromosomal *vanC* locus[6,7]. A fourth gene, called *vanD*, is found in strains resistant to various levels of vancomycin and teicoplanin[8]. Recently, an *Enterococcus faecalis* strain has been described which contains a novel *vanE* gene that was resistant to low levels of vancomycin (minimum inhibitory concentration [MIC] 16 mg/L) and was susceptible to teicoplanin[9].

This report describes the isolation and partial characterization of the first Canadian *E. faecalis* strain harbouring *vanE*.

Methods

Phenotypic characterization: Standard biochemical tests were carried out for strain identification[10]. Initial identification and susceptibility testing was conducted using a Vitek® (GPI cards, bioMérieux, Hazelwood, Missouri). Antimicrobial susceptibilities were confirmed using agar dilution according to NCCLS* guidelines[11], using penicillin, ampicillin, gentamicin, streptomycin, tetracycline, doxycycline, chloramphenicol, vancomycin, and teicoplanin.

DNA Methodology: Genomic DNA was extracted from enterococci and polymerase chain reaction (PCR) was carried out using primers specific to *vanA, vanB, vanC, vanD, vanE, ddl*$_{E.\ faecium}$, and *ddl*$_{E.\ faecalis}$ genes as previously described [8,9].

Computer-aided analysis: Homology searches were carried out using the BLAST suite of programs via the World Wide Web interface of the National

Center for Biotechnology Information <http://www.ncbi.nlm.nih.gov>.

---

* The acronym used to stand for "National Committee for Clinical Laboratory Standards," but NCCLS is now a global organization and develops consensus documents for additional audiences beyond the clinical laboratory community. Therefore, the organization is now referred to only by the acronym, "NCCLS."

Results

Patient History: A wound swab was submitted from a woman, 56 years of age, from Northern Manitoba. The patient had a medical history of hypertension, Raynaud's phenomenon, osteoarthritis, obesity, asthma, and leg ulcers due to poor venous circulation. There was no history of diabetes, cancer, recent antibiotic or steroid use, or other immunosuppressed conditions. The patient presented on 7 June, 2000, to an outpatient clinic with a 5 month history of a one cm crusted skin lesion of the right medial malleolus (ankle) that had become painful the night before. The lesion was excised on 9 June, and found to be a benign ulcer on histopathologic examination. The excision site failed to heal and 3 weeks later the patient returned with erythema, pain, and purulent discharge at the excision site. The site was swabbed for bacterial culture and sensitivity, and she was empirically started on oral cloxacillin, which resulted in no improvement of the infected ulcer. Subsequent treatment with oral amoxicillin resulted in slow resolution of the signs and symptoms over the ensuing 2 weeks.

Further history revealed no travel outside of Manitoba for 20 years and no entertainment of out-of-province visitors. The patient owned no pets and was not in contact with animals. The patient worked as a cook in a fast food restaurant and recalled no trauma to the ankle. She owned a greenhouse and kept only chemical fertilizers there. Her last hospitalization had been in Manitoba, at a local community hospital where she had been admitted for 3 days for pneumonia in November of 1999. Oral cefuroxime axetil had been administered. No screening swabs were collected at that time.

Laboratory Results: A wound swab was submitted on 14 July, 2000 to Cadham Provincial Laboratory in Winnipeg, Manitoba and planted to routine plated media. Within 24 hours a light amount of *Staphylococcus simulans* (oxacillin resistant, but susceptible to all other tested antibiotics, including vancomycin) and a moderate amount of *E. faecalis* grew that was resistant only to quinupristin/dalfopristin according to the Vitek® system used. A 6 mg/mL vancomycin screen plate showed a fine growth after 24 hours that was identified as an *E. faecalis* which was intermediately susceptible to vancomycin and fluoroquinolones. MIC determination using E-Test revealed a MIC to vancomycin of 24 mg/mL. Multiplex PCR for *vanA*, *B*, and *C* was inconclusive [12]. The isolate was submitted to the Nosocomial Infections Laboratory of the National Microbiology Laboratory, Health Canada for further analysis. PCR analysis was negative for the *vanA*, *vanB*, *vanC*, and *vanD* genotypes, however, a product was obtained of the correct size using primers specific to the recently described *vanE* gene[9]. The amplicon was purified and sequence analysis revealed 96% identity at the nucleotide level to *vanE*. Seven of the 21 observed changes resulted in five amino acid changes in the predicted protein. The two proteins are 97% identical.

Discussion

To date, there has only been one other reported case of a *vanE*. The *E.*

faecalis was isolated from the peritoneal dialysis fluid of a patient with peritonitis from Chicago, Ill., who previously received vancomycin[9]. The deduced amino acid sequence of *vanE* was shown to be more closely related to *vanC* (55% identity) as compared to *vanA* (45%), *vanB* (43%), or *vanD* (44%) suggesting the genetic organization of the *vanE* operon more closely resembles the *vanC* operon[9]. It is interesting to note that the Canadian *vanE* isolate was identified from a patient with no travel history, which is very similar to the situation surrounding the first Canadian *vanD* isolate[8]. The origin of this type of resistant organism is unknown, however, the complex nature of the genetics of vancomycin resistance suggests the strain acquired the resistance through lateral gene transfer. Studies are underway to elucidate the structure of the *vanE* operon in this Canadian isolate and to determine if the *vanE* genotype is transferable.

Conclusions

Canadian laboratories should be aware that strains displaying intermediate resistance to vancomycin may harbour the *vanE* gene. PCR using the primers, recently described by Fines (1999), should be used in any *vanA*, *vanB, and vanD* PCR-negative isolates displaying this phenotype.

Acknowledgements

The authors thank the expert eyes of Anna Lutyj, the technologist who picked up the difficult-to-see fine growth.

References

1. Kibsey PC, Willey B, Low DE et al. *Vancomycin-resistant **Enterococcus faecium**: first Canadian isolate*. 61st Conjoint Meeting of Infectious Diseases. 1993. Canadian Association for Clinical Microbiology and Infectious Diseases, Vancouver, November 8-10. (Abstract K5).

2. Lior L, Litt M, Hockin J et al. *Vancomycin-resistant enterococci on a renal ward in an Ontario hospital.* CCDR 1996;22:125-28.

3. Conly J, Ofner-Agostini ME, Paton S et al. *The emerging epidemiology of vancomycin resistant enterococci in Canada: Results of the Canadian Nosocomial Infection Surveillance Program Passive Reporting Network.* 1997. 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, September 28-October 1. (Abstract #E-129B).

4. Arthur M, Molinas C, Depardieu F et al. *Characterization of Tn1546, a Tn-3-related transposon cinferring glycopeptide resistance by synthesis of a desipeptide petidoglycan precursor in **Enterococcus faecium** BM4147.* J Bacteriol 1993;175:117-27.

5. Quintiliani R, Courvalin P. *Characterization of Tn1547, a composite transposon flanked by IS16 and IS256-like elements, that confers vancomycin resistance in **Enterococcus faecalis** BM4281.* Gene 1996;172:1-8.

6. Dutka-Malen S, Molinas C, Arthur M et al. *Sequence of the vanC gene of **Enterococcus gallinarum** BM4147 encoding a D-alanine: D-alanine ligase-related protein necessary for vancomycin resistance.* Gene 1992;112:53-58.

7. Navarro F, Courvalin P. *Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in **Enterococcus casseliflavus** and **Enterococcus flavescens**.* Antimicrob Agents Chemother 1994;38:1788-93.

8. Boyd DA, Conly J, Dedier H et al. *Molecular characterization of the vanD gene cluster and a novel insertion element in a vancomycin-resistant **Enterococcus** isolated in Canada.* J Clin Microbiol 2000;38:2392-94.

9. Fines M, Perichon B, Reynolds P et al. *VanE, a new type of acquired glycopeptide resistance in **Enterococcus faecalis** BM4405.* Antimicrob Agents Chemother 1999;43:2161-64.

10. Facklam RR, Sahm DF. *Enterococcus*. In : Murray PR, Baron EJ, Pfaller MA et al. eds. *Manual of Clinical Microbiology,* 6th edition. ASM Press, Washington, DC. 1995:308-14.

11. NCCLS. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*; Approved Standard - 5th edition. NCCLS document M7-A5.NCCLS: Wayne, Pennsylvania, 2000.

12. Dutka-Malen S, Evers S, Courvalin P. *Detection of glycopeptide genotypes and identification to the species level of clinically relevant enterococci by PCR*. J Clin Micro 1995;33:24-27. Erratum 1995;33:1434.

Source:
P Van Caeseele, MD, S Giercke, RT, J Wylie, PhD, Cadham Provincial Laboratory; D Boyd, BSc MSc, M Mulvey, PhD, National Microbiology Laboratory, Nosocomial Pathogens Division, Winnipeg; S Amin, MB ChB, Flin Flon Clinic, Flin Flon, Manitoba; M Ofner-Agostini, MHSc, BscN, Centre for Infectious Disease Prevention and Control, Health Canada, Toronto, Ontario.

[Previous] [Table of Contents] [Next]