Case Report

Leuconostoc garlicum: An Unusual Pathogen in the Era of Vancomycin Therapy

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ABSTRACT

Leuconostoc garlicum, belonging to the family of *Leuconostocaceae,* is a catalase-negative, Gram-positive ovoid cocci, intrinsically resistant to vancomycin. Clinical infection by *Leuconostoc garlicum* is rare. We report a case of respiratory tract infection subsequent to vancomycin therapy. **[Indian J Chest Dis Allied Sci 2012;54:127-130]**

Key words: *Leuconostoc garlicum,* Pulmonary infection, Vancomycin.

INTRODUCTION

Leuconostoc is, along with other lactic acid bacteria, such as *Pediococcus* and *Lactobacillus*, responsible for causing the unpleasant odour when creating a sourdough starter. The genus *Leuconostoc* includes catalase negative Gram-positive cocci arranged in pairs and in chains. These may appear cocco-bacillary when grown in thioglycollate broth. All species within this genus are heterofermentative and are able to produce dextran from sucrose.[1] These are facultative anaerobes and are inherently resistant to vancomycin. These are commonly found in natural environments, like vegetables and roots that forms their ecological niche. These are also found normally in milk, dairy products and other fresh fruits and find commercial application in production of wine cheese and sugar. These are usually non-pathogenic, though a few species have been considered pathogenic in humans.[2] Previous studies[3,4] have documented that *Leuconostoc* are not part of normal human flora.

CASE REPORT

A 17-year-old female was admitted with a history of unresolving bilateral lower lobe consolidation, right-sided pleural effusion and mediastinal adenopathy. She was started on antituberculosis chemotherapy after the aspirated pleural fluid on analysis was found to be rich in lymphocytes and exudative in nature with a raised adenosine deaminase (ADA) level. As her clinical condition worsened, vancomycin was added to the existing therapy. Fiberoptic bronchoscophy was carried out two days later. The bronchoalveolar lavage (BAL) grew small alpha haemolytic colonies (Figure 1) of catalase-negative, non-motile, Gram-positive cocci (Figure 2) on sheep blood agar that were resistant to vancomycin but sensitive to all other classes of antibiotics.

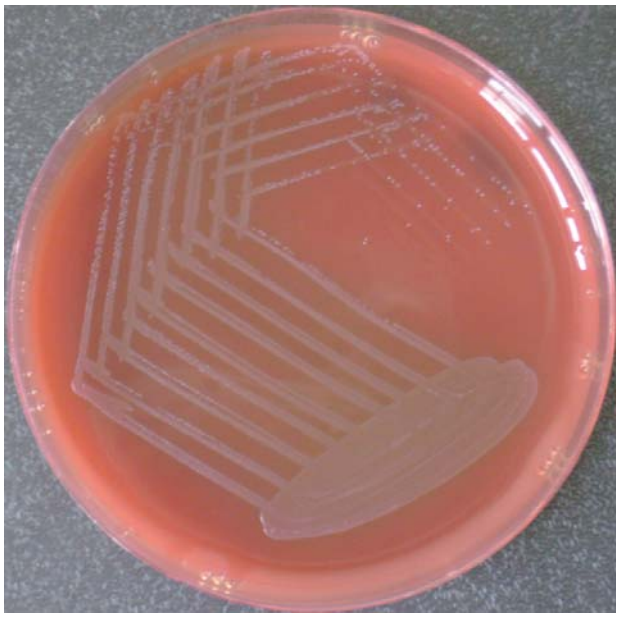

Figure 1. Photograph showing small grey alpha haemolytic clonies of *Leuconostoc garlicum* on sheep blood agar.

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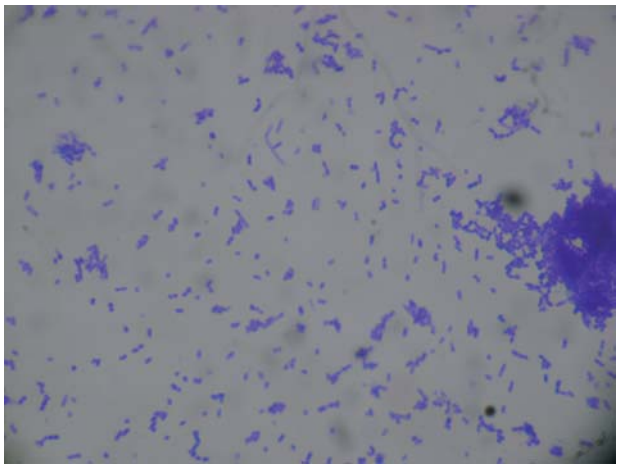

Figure 2. Photomicrograph showing Gram-positive cocci on sheep blood agar (x100).

Gram stain morphology from thioglycollate broth cultures revealed Gram-positive cocco-bacilli (Figure 3). Further biochemical testing revealed that growth was negative for pyrolidonyl arylamidase (PYR), leucine arylamidase (LAP), cytochrome oxidase and arginine dihydrolase (ADH) while esculin hydrolysis in the presence of bile was positive.

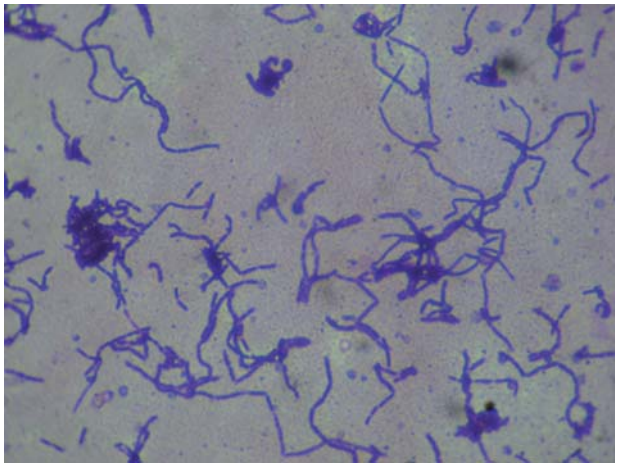

Figure 3. Photomicrograph showing Gram-positive cocco-bacilli on thioglycollate broth (x100).

Acid and carbon dioxide (CO_2) production was seen from glucose in de Man, Rogosa and Sharpe (MRS) broth, ruling out *Streptococci*, *Pediococcus* and *Lactobacillus* species. Acid was produced from raffinose, galactose and xylose while it was negative for arabinose. *Weissella* spp also show identical biochemical and Gram stain morphology when compared to *Leuconostoc* spp except for the fact that the former are ADH positive. Minimum inhibitory concentration estimated by E-test (AB Biodisk, Solona, Sweden) for vancomycin was >256mg/L and for daptomycin was 0.125mg/L (sensitive). Vancomycin was discontinued and carbapenems were started. Pleural fluid and bone marrow cultures were negative. The patient subsequently developed respiratory failure and had to be intubated. Later chest radiographs showed widespread bilateral involvement and endotracheal aspirate grew only multi-drug resistant *Acinetobacter baumanii*, sensitive only to colistin and tigecycline that were promptly started. Since *Leuconostoc garlicum* was not isolated in subsequent cultures its pathogenicity remains questionable. The condition of the patient worsened developing metabolic acidosis and she succumbed to a massive cardiac arrest on the 23rd day of hospitalisation.

The need to confirm the identity of this rare pathogen, prompted us to sequence the 16S rRNA of the organism. Cells used for deoxyribonucleic acid (DNA) extraction were grown in Luria-Bertani medium for 16 hours at 30°C. It was extracted using acid guanidinium thiocyanate-phenol-chloroform extraction method.[7] The 16S rRNA cistrons of this isolate were amplified with the bacterial universal primers 27F (8 to 27, forward; 5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (1,492–1,510, reverse; 5′-GGTTACCTTGTTACGACTT-3′).[8] Partial sequence of the polymerase chain reaction products was determined in an Applied Biosystems Prism cycle sequencing kit (BigDye terminator cycle). The sequence was compared with GenBank entries using BLAST (available from: URL: http://www.ncbi.nlm.nih.gov/ BLAST), that indicated 100% similarity to prototype strain sequence (HM803936.1) *Leuconostoc garlicum* strain B/C-2. *The 190 bp sequence of the 16S rDNA determined in this study was deposited in the GenBank database under accession number HQ433524.*

DISCUSSION

The pathogenic potential of *Leuconostoc* spp was first recognised by Buu-Hui and co-workers[9] in 1985 when it was isolated from an immunocompromised patient. Since then these have been implicated in a variety of infections, particularly in patients with a wide range of underlying disorders, such as, alcoholic liver disease, chronic renal failure patients on dialysis, infants with short bowel syndrome, patients with central venous catheter and patients on enteral feeding.[10] Only five species of *Leuconostoc,* namely, *L. mesenteroides* spp *mesenteroides, L. mesenteroides* spp *dextranicum, L. mesenteroides* spp *cremoris, L. pseudomesenteroides, L. paramesenteroides, L. lactis* and *L. citreum* are currently considered as human pathogens.[1] They are inherently resistant to vancomycin due to the presence of L-alanyl-L-alanine instead of D-alanyl-D-alanine to which the drug binds.[9]

Prior vancomycin therapy is an important risk factor and was observed in 64% of all pediatric case reports of *Leuconostoc*.[11] Many authors[2] have postulated

that the portal of entry may be either skin or gastrointestinal tract, especially as most of the cases were associated with gastrointestinal pathology.[2] The actual disease burden cannot be estimated as identification of *Leuconostoc* spp using routine biochemical tests (Table 1) is labour intensive and time consuming, and in the absence of vancomycin testing these are likely to be wrongly classified as *viridans streptococci*. Automated identification systems, on the other hand, may overdiagnosis as many isolates of *Streptococcus*, *Pediococcus*, *Lactobacillus* and *Weissella* have been labelled as *Leuconostoc* spp.[5] Therefore, molecular methods including sequencing seem to be the gold standard for identification and speciation of this genus.

Table 1. Microbiological characteristics of *Leuconostoc* spp[5,6,12,13]

Test	Result
Gram staining (Blood agar)	Gram positive cocci
Gram staining (Thioglycollate broth)	Gram positive cocco-bacilli/cocci
Catalase	Negative
Leucine arylamidase	Negative
Arginine dihydrolase	Negative
Pyrolidonyl arylamidase	Negative
CO_2 production from glucose in MRS* broth	Positive
Bile esculin	Variable

*=de Man, Rogosa and Sharpe; CO_2=Carbon dioxide

Leuconostoc garlicum as the name suggests was first isolated from garlic in 2002. Subsequently in 2006 and 2009 two cases of bacteraemia were reported.[12,13] Our search of literature revealed only four case reports of *Leuconostoc* respiratory infections (Table 2).[2,14,15] None of them have reported *Leuconostoc garlicum* as the causative agent. Both had vancomycin therapy as a risk factor and had invasive haemodynamic monitoring. As it was isolated two days after initiating vancomycin therapy in the present case, it suggests that antibiotic selection pressure was responsible for the infection. Consumption of a liquid diet in recent weeks, predominantly comprising of milk and fruit juices which are known to harbor *Leuconostoc* spp naturally may here contributed. Contaminated infant feed and enteral feeding related blood stream infections due to *Leuconostoc* spp have been documented before.[15,16]

Frequent use of vancomycin to treat serious infections in hospitalised patients has led to a selection of vancomycin resistant Gram-positive organisms. Vancomycin resistant Gram-positive cocci merit close examination, *Streptococci*, *Pediococcus* and *Lactobacillus* species can be ruled out by testing for gas production from glucose in MRS broth and as a rule *Leuconostoc* spp are LAP and ADH negative. Broth Gram stain morphology shows Gram-positive coccobacilli ruling out enterococci and ADH negativity differentiates it from *Weissella* spp. Awareness of this emerging pathogen is essential for optimal management, as vancomycin monotherapy is a failure. Fortunately, a wide choice of antibiotics is available for treatment. Although the bacteria is sensitive to all other antibiotics, the presnt case was complicated by *Acinetobacter* infaction that ultimately proved fatal.

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Table 2. Reported cases of pulmonary infections by *Leuconostoc* spp in the literature

Authors and Year[ref.]	Age (in years) and Gender	Underlying Condition/Risk Factor	Specimen	*Leuconostoc* spp	Identification Method	Treatment/Outcome
Giacometti *et al*, 1993[6]	33/M	AIDS	Lung	*Leuconostoc citreum*	Biochemical	Teicoplanin/Expired
Borer *et al*, 1997[14]	46/F	RHD, Pneumonia, Vancomycin	Pleural fluid	*Leuconostoc* spp	Biochemical	Clindamycin/ Recovered
Camarasa *et al*, 2009[2]	75/M	COPD, Pulmonary abscess	Pus, BAL	*Leuconostoc* spp	Biochemical	Cefditoren Pivoxil/ Recovered
Ferrer *et al*, 1995[15]	31/M	AIDS, Pneumonia	BAL	*Leuconostoc* spp	Biochemical	Amoxicillin-Clavulanic acid/ Recovered

M=Male; F=Female; AIDS=Acquired immunodeficiency syndrome; RHD=Rheumatic heart disease; COPD=Chronic obstructive pulmonary disease; BAL=Bronchioalveolar lavage

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