Leuconostoc mesenteroides cause Nosocomial UTI At a tertiary care center in North India

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Abstract

Eight strains of gram-positive cocci highly resistant to vancomycin (MICs of 512 - 1,024 µg/ml) were isolated from urine sample. These organisms were identified as *Leuconostoc mesenteroides*. They were tested to determine susceptibility to 12 antimicrobial agents by agar dilution method. Out of 8 isolates of *L. mesenteroides*, all of these isolates (100%) were resistant to vancomycin and 6 (75%) were resistant to teicoplanin. Seven (87.5%) isolates were sensitive to clindamycin, 6 (75.0%) isolates were sensitive to chloramphenicol, erythromycin, gentamicin and streptomycin, 5 (62.5%) isolates were sensitive to ampicillin, penicillin G and tetracycline, while 4 (50%) isolates were sensitive to kanamycin and Trimethoprim. We are reporting nosocomial UTI caused by these organisms. The results provide evidence for the possibility of nosocomial transmission of this unusual opportunistic, vancomycin-resistant pathogen.

Introduction

Leuconostoc species are gram-positive catalase-negative coccoid or coccobacillary (Facklam and Elliott, 1995). In 1985, Buu-Hoi *et al*. (1985) reported the first cases of *Leuconostoc* infection in humans. Since then, *Leuconostoc* spp. have been implicated in a variety of infections (Handwerger *et al*., 1990; Ferrer *et al*., 1995; Jimenez-Mejias *et al*., 1997; Cappelli *et al*., 1999; Albanese *et al*., 2006), particularly in patients being treated with vancomycin and in immunocompromised patients (Albanese *et al*., 2006). *Leuconostoc* species are often misidentified as lactobacilli, streptococci, pediococci or enterococci as all these genera share several biochemical properties (MacGowan *et al*., 1989; Winston *et al*., 2004).

The *Leuconostoc* are naturally highly resistant to vancomycin with MIC>256 µg/ml but could be successfully treated with penicillin with MIC ranging from 0.25 -1.0 unit/ml (Buu-Hoi *et al*., 1985; Kulwichit *et al*., 2007).

In the last decade Leuconostoc species have been reported with increasing frequency as human pathogens, causing bacteremias (Barreau C, Wagener, 1990), meningitis (Friedland *et al*., 1990), breast abscess (Barry *et al*., 1993), abdominal abscess (Montejo *et al*., 2000), peritonitis (Templin *et al*., 2001: Helali *et al*., 2005). Occasionally it has been isolated in cases of catheter associated infections, sepsis, pneumonia, osteomyelitis and hepatic dysfunction (Jofré *et al*., 2006). Recently, these organisms have also been implicated in causing a small outbreak of nosocomial urinary tract infection UTI. The aims of the present study were to characterize the epidemiologic features of the infection with *L. mesenteroides*.

Methods

Sample collection and identification

A total of 558 urine samples (midstream and catheter) were recovered for a period of 5 months from January to June 2008. The samples were submitted for

culture to the Department of Medical Microbiology at Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India, from admitted patients for suspected UTI, and cultured on cysteine lactose electrolyte deficient medium (CLED) (Difco Laboratories, Detroit, USA) during the period. Suspected *Leuconostoc* colonies were streaked onto brain heart infusion (BHI) agar (Difco Laboratories, Detroit, USA) supplemented with 6 µg/ml vancomycin and incubated overnight at 37^{O}C. Only the initial isolate from each patient was tested.

Biochemical characteristics

Tentative identification of bacterial colonies belonging to *L. mesenteroides* was performed by culture characteristics, Gram stain, catalase test, ability to hydrolysis of esculin using bile esculin azide (BEA) broth (Difco Laboratories, Detroit, USA), ability to utilize different carbon sources and production of gas from glucose, ability to grow in BHI agar containing 6.5% NaCl and growth in BHI broth at incubation temperature of 10^{O}C as per conventional identification scheme (Facklam *et al*., 1989). Vancomycin susceptibility was tested by E-test (AB Biodisk, Solna, Sweden). Definitive identification up to species level was made with the BD-BBL Crystal Identification System, (Gram-Positive ID Kit-Sparks, Maryland, USA).

Antimicrobial susceptibility testing

L. mesenteroides strains were tested for resistance to ampicillin, penicillin G, erythromycin, chloramphenicol, clindamycin, gentamicin, kanamycin, streptomycin, tetracycline, trimethoprim, vancomycin and teicoplanin according to the current guidelines of (NCCLS, 2000). Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard. A 10.0 µl aliquot of each suspension was spotted onto the agar surface to achieve a final inoculum of approximately 10^5 CFU per spot. It was allowed to absorb into the agar for 10 min. The results were read after incubation at 37^{O}C for 18-24 h and 48 h. Plates were read against a dark,

nonreflecting background. Strains were considered antibiotic resistant if growth was positive or weak or if more than one colony was observed.

Results

Biochemical identification.

Eight *L. mesenteroides* isolates were recovered from 8 patients (aged 2 months to 65 years) were gram-positive cocci occurring in pairs or short chains, all of them are highly resistant to vancomycin (MICs 512 - 1,024 µg/ml). The biochemical analysis by the BD-BBL Crystal Identification System are shown in Table 1.

Table 1. Characteristics for identification of *L. mesenteroides* by BD BBL CRYSTAL™ system.

Test	**Substrate**	***L. mesenteroides***
Morphology	Cocci	+
	Coccobacilli	+
	Rod	+
Arrangement	Chains	+
	Pairs	+
	Clusters	-
Gas from	Trehalose	+
	Lactose	V
	Galactose	-
	Mannitol	-
	Maltose	+
	Arabinose	+
	Raibose	-
	Raffinose	+
	Esculin	+
	Arginine	-
	PYRase	-
Growth	at 10^{O}C	+
	at 45^{O}C	-
	in 6.5% NaCl	+
	Methyl red	-
	Voges-Proskauer	V
Resistant to	vancomycin	512 - 1,024 µg/ml

Antimicrobial susceptibility testing

The susceptibility testing results of 8 *L. mesenteroides* strains against 12 antimicrobial agents are shown in (Table 2).

Table 2. Susceptibility of 8 strains of *Leuconostoc mesenteroides* to 12 antimicrobial agents.

Antibiotics	No of resistance / 8	(100%)
Ampicillin	3	(37.5)
Chloramphenicol	2	(25.0)
Clindamycin	1	(12.5)
Erythromycin	2	(25.0)
Gentamicin	2	(25.0)
Kanamycin	4	(50.0)
Penicillin G	3	(37.5)
Streptomycin	2	(25.0)
Teicoplanin	6	(75.0)
Tetracycline	3	(37.5)
Trimethoprim	4	(50.0)
Vancomycin	8	(100)

Clinical details

The clinical details for 8 patients (5 females and 3 males) are shown in (Table 3). The age ranged from 2 months to 65 yr. All females were admitted in the obstetrics and gynaecological unit. Three of them suffered from catheter related nosocomial UTI and responded to removal of catheter plus antibiotics. In two females there were no symptoms related to UTI and therefore the organisms were considered as contaminants. Among the three male patients who had nosocomial UTI, one had malignancy of urinary bladder and another had stricture urethra. The third was a two-month old child with exstrophy bladder. All patients responded to antibiotics. None of the patients developed bacteremia.

Table 3. Clinical Profile of Patients With Significant Bacteriuria Due to *Leuconostoc mesenteroides*

Patients	Age / Gender	Ward	Clinical diagnosis	Catheter/Urinary Instrumentation	Type of UTI	Treatment
1	25y/F	Obstetrics & Gynecology	Preterm labor	Yes	Nosocomial UTI	Amoxycillin
2	43y/F	=	Surgery for Fibroid uterus with endometriosis	Yes	=	Ciprofloxacin & gentamicin & removal of catheter
3.	25y/F	=	Preterm labor	No	Asymptomatic	-
4	55y/F	=	Surgery for Ovarian tumor	Yes	Nosocomial UTI	Gentamicin & removal of catheter
5	27y/F	=	Preterm labor	No	Asymptomatic	-
6	65y/M	Surgery	Surgery for Urinary bladder	Yes	Nosocomial UTI	Ciprofloxacin
7	60y/M	Urology	Stricture urethra	Yes	=	Augmentin & amikacin
8	02m/M	Pediatric surgery	Exstrophy bladder	No	=	Penicillin & Gentamicin

F, female; M, male; y, year; m, month

Discussion

L. mesenteroides is one of several uncommon, gram-positive, intrinsically vancomycin-resistant bacteria, that can cause serious human infections (Facklam and Elliott, 1995; Ferrer *et al*., 1995; Moellering, 1995). *Leuconostoc* species are difficult to detect with routine methods (Facklam and Elliott, 1995) and can easily be misidentified as *Lactobacillus*, alpha-hemolytic streptococci, *Pediococcus*, *Enterococcus*, or *Lactococcus* (Facklam and Elliott, 1995). It must be distinguished from other mentioned species as there share almost similar biochemical properties (Facklam *et al*., 1989; Isenberg *et al*., 1988; Riebel and Washington, 1990; Barreau and Wagener, 1990).

A total of 558 urine samples were cultured on (CLED) medium. The *Leuconostoc* were incidentally found. These species we observed were more coccobacillary than coccal in shape. All the strains were high resistant to vancomycin (MIC>1024µg/ml). in 1984, Shlaes and his co workers suggested that gram-positive bacteria should be routinely tested for vancomycin susceptibility. Vancomycin-resistant gram-positive cocci merit close examination, since both conventional tests and the API 20 Strep System can lead to misidentification of Lactobacillus spp. and of strains belonging to the genus Leuconostoc as viridans streptococci (Thornsberry *et al*., 1984). In our experience, Leuconostoc spp. can be suspected from the vancomycin resistance and the gas production. All the strains are catalase-negative, PYR negative, could grow in presence of 6.5% Nacl and at 10^{o}C. All produced acid from sucrose, maltose, raffinose, arabinose, and trehalose. In the present study, 6 out of 8 were lactose-positive and all were Mannitol-negative. None of the strains deaminated arginine. In the BD-BBL Crystal Identification System, none of the Leuconostoc strains were positive for the PRYase test. The strains were identified as *L. mesenteroides* based on conventional scheme of Facklam *et al*., (1989).

Knowledge of the susceptibility of *L. mesenteroides* should help physicians treat infections caused by these strains. Many of the antimicrobial agents that we tested did not have uniform activities against the *L. mesenteroides* tested, so it appears that proper

identification will help in the formulation of optimal antimicrobial regimens. In the present study, the results of the antibacterial activities of the various antibiotics showed that all *L. mesenteroides* isolates were resistant to vancomycin, while 6/8 of *L. mesenteroides* isolates were resistant to teicoplanin; our study is similar to the study of (de la Maza *et al*., 1989; Albanese *et al*., 2006).

The present study's results showed that, out of 8 isolates of *L. mesenteroides*, 7 were susceptible to clindamycin, 6 *L. mesenteroides* strains were susceptible to chloramphenicol, erythromycin, gentamicin and streptomycin. While 5 isolates were susceptible to ampicillin, penicillin G and tetracycline, and 4 *L. mesenteroides* isolates were susceptible to kanamycin and Trimethoprim. Our results are a slightly similar to the observation of Buu-Hoi *et al*., (1985) who recognized that Penicillin G and ampicillin were more active. While all strains were susceptible to tetracycline and chloramphenicol. Erythromycin and clindamycin were highly active against all strains. Trimethoprim is not active. All strains were susceptible to gentamicin. While, both of streptomycin and kanamycin were less active (Buu-Hoi *et al*., 1985). Several reports have found that those with the best activities against all the *L. mesenteroides* strains tested were clindamycin, chloramphenicol, gentamicin, erythromycin, penicillin and tetracycline (Swenson *et al*. 1990; Bou *et al*., 2008).

As *L. mesenteroides* are intrinsically resistant to vancomycin, infections occur more frequently in patients being treated for underlying diseases with vancomycin therapy (Bauer *et al*., 1966). None of the patients in the present study had received vancomycin. Therefore, though uncommonly isolated (8/558-<0.014%) organisms can cause nosocomial UTI and have the potential for causing an outbreak. the study of Bou *et al*., (2008) have demonstrated that out of 42 patients infected with *L. mesenteroides*, 9 of the patients died, and 3 of the deaths (7.1%) were directly related to the *Leuconostoc* infection. In addition to these cases, a vancomycin resistant viridans group streptococcus reported by Shlaes *et al*., 1984 may actually have been a *Leuconostoc* sp.(Isenberg *et al*., 1988). This report is further evidence that, although rarely pathogenic, leuconostocs may cause severe infection in humans.

In summary, though uncommonly isolated these organisms can cause nosocomial UTI and have potential for causing outbreak. These are likely to be misidentified as enterococci in routine microbiology laboratory.

Conclusions

The Leuconostoc species has been considered as a potential pathogen especially in the immunocompromised host. Its clinical significance in other patients may be questionable. Further work need to be done to determine if the Leuconostoc species are part of the skin flora and thus give rise to contaminated urine culture results when the skin is not adequately cleaned prior to urine sampling. It would be also necessary to determine the conditions in which these isolates would be of clinical significant.

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Leuconostoc mesenteroides المسببة لاصابات المستشفى لالتهاب القناة البولية في مركز التعليم العالي للرعاية في شمال الهند

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الخلاصة

عزلت ثمان عزلات من المكورات الموجبة لصبغة كرام المقاومة للمضاد الحيوي الفانكومايسين (بتركيز مثبط ادنى > ١.٠٢٤ مايكروغرام/مل) من عينات الادرار. شخصت هذه العزلات *Leuconostoc mesenteroides* .اختبرت حساسيتها تجاه ١٢ مضاد حيوي بطريقة تخافيف الاكار. جميع هذه العزلات (١٠٠ ٪) اظهرت مقاومة للفانكومايسين و٦ عزلات (٧٥ ٪) مقاومة للتيكوبلانين. بينما كانت ٧ عزلات (٨٧.٥ ٪) حساسة تجاه كلندامايسين، ٦ عزلات(٧٥ ٪) حساسة تجاه الكلورومفينيكول، الارثرومايسي، الجنتامايسن والستربتومايسين. ابدت ٥ عزلات (٦٢.٥ ٪) حساسية تجاه الامبسلين، البنسلسن ج والتتراسايكلين. بينما اظهرت ٤ عزلات (٥٠ ٪) حساسية تجاه الكانامايسين والترايميثبريم. تم تسجيل اصابات المستشفى المتسببة عن هذه البكتريا. هذه النتائج قدمت ادلة على امكانية انتقال اصابات المستشفى عن طريق هذه البكتريا الانتهازية غير الاعتيادية.