ISSN 2219-2808 (online)

World Journal of *Clinical Pediatrics*

World J Clin Pediatr 2020 November 19; 9(3): 44-62

Published by Baishideng Publishing Group Inc

WJCP

World Journal of Clinical Pediatrics

Contents

Irregular Volume 9 Number 3 November 19, 2020

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

World Journal of Clinical Pediatrics

Irregular Volume 9 Number 3 November 19, 2020

Contents

NAME OF JOURNAL
World Journal of Clinical Pediatrics

ISSN
ISSN 2219-2808 (online)

LAUNCH DATE
June 8, 2012

FREQUENCY
Irregular

EDITORS-IN-CHIEF
Toru Watanabe, Consolato M Sergi

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2219-2808/editorialboard.htm

PUBLICATION DATE
November 19, 2020

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2020 November 19; 9(3): 44-52

DOI: 10.5409/wjcp.v9.i3.44

ISSN 2219-2808 (online)

MINIREVIEWS

Influenza B infections in children: A review

Yellanthoor Ramesh Bhat

ORCID number: Yellanthoor Ramesh Bhat 0000-0003-2919-6361.

Author contributions: Bhat YR conceptualized the review topic and wrote the manuscript.

Conflict-of-interest statement: The author(s) declare no conflicts of interest related to this article.

Manuscript source: Invited manuscript

Specialty type: Pediatrics

Country/Territory of origin: India

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): D

Yellanthoor Ramesh Bhat, Department of Pediatrics, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India

Corresponding author: Yellanthoor Ramesh Bhat, MBBS, MD, Doctor, Full Professor, Department of Pediatrics, Kasturba Medical College, Manipal Academy of Higher Education, Madhava Nagar, Manipal, Udupi District, Karnataka 576104, India.
docrameshbhat@yahoo.co.in

Abstract

Influenza B (IFB) virus belongs to the Orthomyxoviridae family and has two antigenically and genetically distinct lineages; B/Victoria/2/87-like (Victoria lineage) and B/Yamagata/16/88-like (Yamagata lineage). The illness caused by IFB differs from that caused by influenza A. Outbreaks of IFB occur worldwide and young children exposed to IFB are likely to have a higher disease severity compared with adults. IFB mostly causes mild to moderate respiratory illness in healthy children. However, the involvement of other systems, a severe disease especially in children with chronic medical conditions and immunosuppression, and rarely mortality, has been reported. Treatment with oseltamivir or zanamivir decreases the severity of illness and hospitalization. Due to the enormous health and economic impact of IFB, these strains are included in vaccines. IFB illness is less studied in children although its impact is substantial. In this review, the epidemiology, clinical manifestations, treatment, prognosis, and prevention of IFB illness in children are discussed.

Key Words: Children; Influenza B; Hospitalization; Oseltamivir; Respiratory infections; Severity

Core Tip: Influenza B (IFB) outbreaks occur worldwide and young children exposed to IFB tend to have a higher disease severity compared with adults. The IFB virus belongs to the Orthomyxoviridae family and has two distinct lineages; Victoria lineage and Yamagata lineage. The illness caused by IFB is less severe than that caused by influenza A. IFB illness is less studied in children although its impact is substantial. IFB mostly causes mild to moderate respiratory illness in healthy children. However, the involvement of other systems, a severe disease especially in children with chronic medical conditions and immunosuppression, and rarely mortality, has been reported.

Grade E (Poor): 0

Received: June 22, 2020
Peer-review started: June 22, 2020
First decision: August 9, 2020
Revised: August 31, 2020
Accepted: September 18, 2020
Article in press: September 18, 2020
Published online: November 19, 2020

P-Reviewer: Borkow G, Komatsu H
S-Editor: Yan JP
L-Editor: Webster JR
P-Editor: Wu YXJ

Early treatment with antiviral agents decreases the severity of illness and hospitalization. Due to the enormous health and economic impact of IFB, these strains are included in vaccines. In this review, the disease burden, clinical manifestations, treatment, prognosis, and prevention of IFB illness in children are discussed.

Citation: Bhat YR. Influenza B infections in children: A review. *World J Clin Pediatr* 2020; 9(3): 44-52
URL: https://www.wjgnet.com/2219-2808/full/v9/i3/44.htm
DOI: https://dx.doi.org/10.5409/wjcp.v9.i3.44

INTRODUCTION

Influenza is a major public health problem worldwide[1-3]. It is one of the most common respiratory infectious diseases. The influenza viruses belong to the Orthomyxoviridae family and contain a single-stranded RNA genome. There are three types of influenza viruses A, B, and C based on different structural arrangements of internal nucleoprotein and matrix protein antigens. Influenza A (IFA) causes more severe illness and hence is well studied. Influenza B (IFB) is less studied in children although its impact is substantial. IFB has two antigenically and genetically distinct lineages, B/Victoria/2/87-like (Victoria lineage) and B/Yamagata/16/88-like (Yamagata lineage). Influenza C (IFC) is known to cause upper respiratory tract infection in younger children. It also causes community-acquired pneumonia. The severity of infection is less than that of IFA but more than that of IFB. C/Kanagawa/1/76-related lineages and C/Sao Paulo/378/82-related lineages are the IFC strains identified in the influenza seasons 2008-2009 and 2009-2010 in Milan[4]. IFB viruses have circulated worldwide since 1983 causing outbreaks now and then[5-8]. IFB usually causes mild to moderate illness in healthy individuals including children. However, severe disease in children, the elderly, and individuals with chronic medical conditions have been reported. Serious illness leading to mortality, although rare, has been described. Worldwide, young children exposed to IFB had a higher disease severity compared with adults[9-13]. Antiviral agents decrease the severity of illness and hospitalization[8]. Due to the enormous health and economic impact of IFB, these strains are included in vaccines. In this review, the disease burden, clinical manifestations, differences between IFA and IFB illness, treatment, prognosis, and prevention of IFB illness in children are discussed.

EPIDEMIOLOGY AND DISEASE BURDEN

IFB was first identified in 1940. The second lineage was discovered in 1983. Since then frequent outbreaks of IFB have occurred worldwide[1,3,5,6]. From 2001 onwards, both IFB lineages have been co-circulating each influenza season. Children are more susceptible to infection. In particular, children under 5 years of age are more susceptible to influenza illness as they are an immunologically naïve population. IFB caused significant morbidity in children in the United States during 2004-2011, and in the United Kingdom during 2010-2011 seasons[1-3,5,6]. The influenza-associated disease burden among infants and young children is likely to be underestimated because of the similarity of illness caused by other respiratory viruses during an influenza season. Annual IFA and IFB attack rates among children aged 5 to 9 years reach 35%, compared with those rates among the adult population which approach 13% for IFA and 6% for IFB. IFB outbreaks occurred worldwide from November 2017 to April 2018. The illness caused significant health and economic impacts worldwide[1-3,5-7].

Mancinelli *et al*[2] reported that among the IFB isolates, 91.54% were the B/Yamagata/16/88 lineage and 8.45% were the B/Victoria/2/87 lineage during the 2012-2013 influenza season in Italy. The B/Yamagata/16/88 lineage was most prevalent in children 3-6 years old. They reported the median length of hospital stay of 3 d for IFB viral illness. An Indian study reported high influenza-associated mortality in children aged less than 5 years in addition to the elderly population[10]. Studies on the incidence, clinical burden, and economic impact of IFB helped to include an additional B strain in the vaccine against influenza. A study involving IFB cases across

9 European countries reported wide variations in IFB cases during 1998-2013[5].

The Global Influenza B Study that included 1820301 influenza cases from 31 countries during 2000-2018 reported 419167 (23.0%) cases of IFB[14]. Cases from the United States (54.1%) and Australia (25.3%) contributed the maximum number of cases. In countries of the Southern hemisphere, IFA epidemics peak in July-September, and IFB peaks in August-September. In countries of Northern hemisphere, IFA peaks in January-February and IFB peaks in February-March (Table 1). However, there were exceptions to this pattern in some countries. In most countries, IFB/Victoria showed a unimodal curve with a peak below 10 years of age. B/Yamagata cases frequently showed two peaks, one peak below 10 years of age, and another peak between 25 and 50 years of age. In tropical countries, the timing of IFA and IFB epidemics differ. There appears to be no consistent pattern in the timing of the different epidemics. B/Victoria occurs more frequently in tropical countries, while B/Yamagata occurs frequently in temperate climate countries of the Southern and Northern hemispheres.

IFB-VIRAL CHARACTERISTICS

IFB viruses belong to the Orthomyxoviridae family[1,5,14]. They are single-stranded RNA viruses. They are classified into two lineages: B/Yamagata and B/Victoria. IFB viruses are further classified into specific clades and sub-clades. The B/Victoria clades include V1A and subclades include V1A.1, V1A.2, and V1A.3. B/Yamagata clades include Y1, Y2, and Y3. There are no subclades in B/Yamagata.

The virion of IFB virus consists of an envelope, a matrix protein, a nucleoprotein complex, a nucleocapsid, and a polymerase complex. The IFB virus has eight genome segments. The proteins of the polymerase complexes include PB1, PB2, and PA. The polymerase complexes are located at the ends of the nucleocapsids. These capsids are helical in nature. The nucleocapsids are encircled by the M1 matrix protein and a lipid bilayer envelope. The viral surface has two glycoproteins; hemagglutinin (HA) and neuraminidase (NA). The HA, NA and the M2 matrix protein are embedded in the lipid bilayer envelope. The IFB virus has 500 or more surface projections. These surface projections are made of HA and NA.

The IFB virus undergoes antigenic variation through genetic reassortment among co-circulating strains and antigenic drift from cumulative mutations[5,14]. IFB viruses generally change their genetic and antigenic properties more slowly than IFA viruses. IFB virus HAs have a mutational rate about five times slower than that observed for IFA virus HAs. The IFB virus is relatively vulnerable to certain environmental impacts. The virus can survive for several hours in the environment depending on humidity and temperature. In water, at < 20°C, the virus can survive for several months. Influenza viruses are sensitive to lipid solvents and detergents.

CLINICAL FEATURES

IFB illness in children ranges from subclinical illness to complicated disease involving multiple organs. The typical manifestations include respiratory tract and systemic signs and symptoms. IFB can present as croup, bronchiolitis, pneumonia and febrile disease mimicking bacterial sepsis. Central nervous system, cardiac, muscle, or renal complications can also occur[1-3,5-7,11-17]. IFB illness in children may predispose to bacterial superinfections. IFB viruses tend to persist across multiple seasons and exhibit complex global dynamics[18]. A peak between August and September has been observed in countries of the Southern hemisphere. In countries of the Northern Hemisphere, the peak occurs in February-March. There are exceptions to this pattern in certain countries. Generally IFB epidemics tend to peak three weeks after the IFA epidemics during winter in temperate countries of Southern and Northern hemispheres[14,18]. An Indian study involving children found more cases from January to May with a peak in March[12]. In younger children distinguishing influenza from other febrile illnesses based on clinical manifestations alone is often difficult.

IFB most often causes respiratory infections with cough, fever, myalgia, chills and malaise. The illness may last for two to eight days. The onset is typically rapid. A minority of patients, especially young children, and those with medical comorbidities may have severe disease. Respiratory and multi-organ failure may occur either due to virus or secondary bacterial pneumonia. IFB also affects children with underlying malignancies such as lymphoma, leukemia, solid tumor, or renal tubular disorders.

The highest frequency of IFB infections is said to occur in infants less than one year

Table 1 The differences between influenza A and influenza B illness in children[2,13-16]

Variables	Influenza A	Influenza B
Epidemiology	Constitutes about 75% of total influenza	Constitutes about 25% of total influenza
Epidemic/pandemic	Causes both epidemics and pandemics	Causes epidemics
Epidemic peak[1]		
Southern hemisphere	July-September	August-September
Northern hemisphere	January-February	February-March
Virology		
Family	Orthomyxoviridae	Orthomyxoviridae
Type	Single-strand RNA virus	Single-strand RNA virus
Subtypes/lineages	18 H subtypes and 11 N subtypes	Two lineages: B (Victoria) and B (Yamagata)
Common types	A(H1N1) and A(H3N2)	Victoria and Yamagata lineages
Pandemic strain	A(H1N1) pdm09 virus	/
Infection	Humans, pigs, horses, wild birds, *etc.*	Only in humans (possibly in seals)
Host factors (children)[13]		
Age (yr) (median)	2.0	4.2
Infants	Commonly affected	Less commonly affected
School-age	Can be affected	Commonly affected
Gender	Male predilection	Male predilection
Clinical features[13,16]		
Fever	High	< 38.5 °C
Febrile convulsion	About 5.4%	About 10.7%
Rhinorrhea	Common	Common
Underlying medical conditions	Not so common (26%)	More common (34%)
Length of hospital stay (median)	5 d	3 d
Gastrointestinal symptoms (abdominal pain, vomiting, diarrhea)	Less common	More common
Myositis	1.1%-6%	4.5%-15%
Otitis media	26%	19%
Laboratory parameters		
Leucopenia	Less common (8%)	More common (19%)
Leucocytosis	8%	7%
Elevated CRP	31%	15%-46%
Treatment		
Oseltamivir	Effective	Effective
Zanamivir inhalation	Effective in ≥ 5 yr of age	Effective in ≥ 5 yr of age
Prognosis	Good	Good
Complications	May occur	In young children/having comorbidities
Vaccine (Quadrivalent)	Effective	Effective

[1]Exceptions do occur in some countries.

of age. The median age of children with IFB viral illness is 4.2 years[13]. High fever especially an abrupt onset is common. Febrile convulsions are reported in 9% of children. Rhinorrhea and cough are the usual manifestations. Vomiting or diarrhea may occur in 25% of cases. Myalgia or myositis may be present in 15% and headache occurs in 25%. Pneumonia, otitis, and encephalitis may occur in a small percentage of children.

An Indian study involving IFB in children found upper respiratory tract infections in 78.5% cases followed by pneumonia in 19.6% and severe pneumonia in 1.7%[12]. The peak of the illness was observed in March. Male children predominated in the study. Male predominance in IFB illness has also been reported in other studies[14-19].

Gastrointestinal symptoms such as abdominal pain, diarrhea, and vomiting in IFB children were reported by Lennon *et al*[16]. Encephalitis is a rare manifestation of IFB. A 6-year-old girl with acute IFB virus encephalitis resulting in neurological sequelae was reported by McCullers *et al*[20]. IFB associated encephalitis, profound weakness, and response to oseltamivir in a 10-year-old boy was reported by Straumanis *et al*[21].

Chi *et al*[19] studied 118 cases of IFB in Taiwan with characteristics of Yamagata and Victoria strains. They reported a higher incidence of lower respiratory tract infections with Yamagata-like strains. All invasive diseases particularly occurred with this strain. Children infected with the Victoria-like group had the longest hospital stays.

HOST FACTORS

Influenza is a major cause of morbidity and mortality in humans globally. Certain age groups are more susceptible to influenza. Children and the elderly are more vulnerable to influenza. IFB commonly affects younger children. Eşki *et al*[22] reported that children less than 5 years of age had a higher hospitalization rate (82.9%). The strongest association between hospitalization and age was observed for children ≤ 2 years of age (63.6%) compared with other age categories (36.4%).

IFB related childhood morbidity and mortality increases with certain comorbidities. Underlying medical comorbidities such as asthma, neurologic deficits, or malignancies were documented in one-fourth of the children with IFA or IFB[13]. Congenital heart disease, neuromuscular disease, immunosuppression, presence of neutrophilia, lymphopenia, severe bacterial infections, and late initiation of antiviral therapy were found to be independent risk factors for prolonged hospitalization in patients with IFB-related lower respiratory infections. Prolonged hospitalization was more common in children with comorbidities (24.3%) compared to children without comorbidities (10.4%).

HOW IS IFB DIFFERENT FROM IFA?

Among influenza viruses, IFA virus causes more severe disease. Certain differences in median age, clinical manifestations, illness severity, risk factors, and length of hospitalization were observed between IFA and IFB illness in various studies[2,6,11,15]. Peltola *et al*[13] reported that the median age of children with IFA was 2.0 years and was 4.2 years in children with IFB. Infants accounted for 27% of children with IFA and 24% of those with IFB. Boys predominated in both IFA and IFB infected children. Underlying medical conditions were present in 26% of the children with IFA and in 34% of those with IFB. Fever is a common symptom in both IFA and IFB affected children. High fever and rhinorrhea were more common in IFA than in IFB. Respiratory symptoms were more prevalent in patients affected by IFA than by IFB.

Mancinelli *et al*[2] reported more IFA cases in children less than one-year-old and more IFB cases among school-age children. The length of stay in children with IFA was significantly longer than those infected with IFB. The median length of stay was 5 d (range: 0-59) for IFA and 3 d (range: 0-116) for IFB. IFB children mostly had a fever < 38°C. An Indian study also observed more IFB cases in the older age group[12]. Respiratory symptoms dominate in IFA children. Lennon *et al*[16] found that gastrointestinal symptoms such as abdominal pain, diarrhea, and vomiting were more common in IFB than IFA infection. Children with IFB were more likely than those with IFA to be diagnosed with upper respiratory tract infection, myositis, and gastroenteritis. Children with underlying malignancies such as lymphoma, leukemia, and solid tumor were more susceptible to IFB infection.

LABORATORY TESTS

Several different approaches are currently available for the diagnosis of influenza infections in children[23-25]. These include nucleic acid amplification tests (NAT), immunochromatography-based rapid diagnostic tests, immunofluorescence assays and viral isolation in cell culture[23]. Rapid molecular assays are the preferred diagnostic tests. The advantages of these tests include accuracy, faster results and ease of the test at the point of care[24].

Rapid molecular assays are more sensitive compared to antigen-based tests. They can detect viruses much earlier in clinical samples. The tests for the diagnosis of influenza infections include reverse transcriptase polymerase chain reaction (RT-PCR), next-generation sequencing, ligase chain reaction, sequencing-based tests including pyrosequencing, DNA microarray-based tests, nucleic acid sequencing-based amplification, loop-mediated isothermal amplification-based assay, simple amplification-based assay, *etc*.[23]. Most of these tests take 2 to 4 h to complete. They demonstrate higher sensitivity and specificity compared with antigen-based tests. RT-PCR, a gold standard assay for influenza diagnosis involves three essential steps: extraction of viral RNA from clinical specimens; reverse transcription of viral RNA to a single-stranded cDNA using the enzyme reverse transcriptase, and amplification of the PCR product is coupled to fluorescent detection of labeled PCR products[23,24]. RT-PCR is the most powerful NAT used to identify influenza viruses across many laboratories in the world.

Nasopharyngeal and nasal swab samples should be obtained from influenza affected children and transported to the laboratory in a universal viral transport medium. Samples of throat and nasal swabs, nasopharyngeal aspirate, bronchoalveolar lavage, and sputum may also be taken depending upon the clinical situations. The samples should either be processed immediately or stored at -80°C before testing. Nucleic acid extraction and reverse transcription should be performed initially. Samples positive for IFB are further characterized by genotyping analysis to identify the subtype; the B/Yamagata/16/88 and B/Victoria/2/87 lineages. Different primers are used to identify the Victoria lineage and Yamagata lineage[23-25].

Other laboratory tests include white blood cell (WBC) counts and serum C-reactive protein (CRP) levels. The WBC counts and CRP levels may be helpful in detecting bacterial co-infections. Both WBC counts and CRP levels are low in uncomplicated IFB illness. A decreased WBC count of less than 4000/mm^3 or more than 15000/mm^3 can occur in approximately 10% of children with IFB[13]. Leukopenia and lymphopenia are common in IFB illness. Hence, these isolated findings need not be considered for further evaluation. IFB is more likely associated with leukopenia than IFA. One study reported an elevated CRP level in 46% of IFB children[2].

TREATMENT

The children affected by IFB need supportive care and antiviral agents[26-30]. The neuraminidase inhibitors are effective for IFB illness in children. Oral oseltamivir is effective in children aged 1-12 years. This drug efficiently metabolizes to the active carboxy metabolite. The oseltamivir carboxylate is primarily eliminated by renal excretion. A pharmacokinetic model in children indicated that a dose of oseltamivir 2 mg/(kg·dose) twice a day would be safe and effective[29].

Early administration of oseltamivir can reduce the risk of IFB virus-associated pneumonia[8]. In-field trials supported the benefit of anti-influenza drugs especially when they are administered within 48 h after the onset of symptoms[13,28,29]. Whitley *et al*[29] in their randomized controlled study enrolled 144 IFB infected children. In the subgroup analyses they showed a significant reduction in the median duration of fever, cough, coryza and other symptoms in the oseltamivir group (Placebo, 100 h; Oseltamivir, 73 h; P = 0.01). Other benefits of oseltamivir include a rapid decline in viral shedding and a significant reduction in complications, particularly otitis media. Oseltamivir is well tolerated in children. Vomiting may be observed in a few children. Oseltamivir treatment might also reduce the likelihood of the spread of influenza to close contacts. Matheson *et al*[30] also found that oseltamivir was effective in reducing secondary complications. Sato *et al*[26] in Japan found that oseltamivir was effective in IFA and IFB, and the benefit was higher among younger children. On the other hand, Suzuki *et al*[27] found that oseltamivir was less effective against IFB than IFA. If clinical suspicion is strong for influenza infection, it is also advisable to start antiviral treatment without waiting for laboratory confirmation in special categories of children;

those with comorbidities and unvaccinated children < 6 mo of age[22].

The main advantages of neuraminidase inhibitors compared with amantadine and rimantadine, are fewer adverse effects, activity against both IFA and IFB, and rare resistance. Zanamivir is another drug effective in shortening the duration and severity of influenza. A 5-d course of twice-daily inhaled zanamivir, 10 mg, was compared with placebo in symptomatic IFB children aged 5 to 12 years[28]. The zanamivir group had a reduction in the duration of IFB symptoms.

PROGNOSIS

Prognosis is generally good for children affected by IFB. Some of the affected children may require hospitalization for 2 to 8 d. The average length of hospital stay of 3 d has been documented[2]. The majority (78.5%) with upper respiratory infections recovered without any complications[12]. The authors reported pneumonia in 14.2% children aged > 5 years, and in 5.3% children aged between one to five years. The disease tends to be severe in young children and those with comorbidities. Mortality due to IFB in children remains low.

VACCINATION

World Health Organization and Centers for Disease Control and Prevention recommend that children between 6 mo and 5 years should be vaccinated against IFA and IFB. Inclusion of this age category in vaccination helps in decreasing hospital admissions for influenza. As there is no licensed influenza vaccine for children younger than 6 mo, alternative strategies including maternal vaccination during pregnancy and household vaccination are likely to reduce the burden of influenza[22]. Multiple influenza vaccine manufacturers have brought out quadrivalent seasonal influenza vaccines consisting of B strain to provide immunity against both lineages of IFB. The influenza vaccine including B strain is likely to offer benefit in children with chronic pulmonary, cardiac, or renal disease, diabetes mellitus, immunosuppression, or in those receiving long-term salicylate treatment.

CONCLUSION

IFB illness affects children worldwide during epidemics. The clinical manifestations vary depending on the lineages. The illness tends to be severe in younger children and those with comorbidities. The antiviral drug, oseltamivir is effective in children especially in reducing the duration of symptoms, viral shedding and secondary complications. The quadrivalent flu vaccine which includes IFB strain is recommended in children.

REFERENCES

1 **Nair H**, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, Simmerman JM, Gordon A, Sato M, Howie S, Krishnan A, Ope M, Lindblade KA, Carosone-Link P, Lucero M, Ochieng W, Kamimoto L, Dueger E, Bhat N, Vong S, Theodoratou E, Chittaganpitch M, Chimah O, Balmaseda A, Buchy P, Harris E, Evans V, Katayose M, Gaur B, O'Callaghan-Gordo C, Goswami D, Arvelo W, Venter M, Briese T, Tokarz R, Widdowson MA, Mounts AW, Breiman RF, Feikin DR, Klugman KP, Olsen SJ, Gessner BD, Wright PF, Rudan I, Broor S, Simões EA, Campbell H. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet* 2011; **378**: 1917-1930 [PMID: 22078723 DOI: 10.1016/S0140-6736(11)61051-9]

2 **Mancinelli L**, Onori M, Concato C, Sorge R, Chiavelli S, Coltella L, Raucci U, Reale A, Menichella D, Russo C. Clinical features of children hospitalized with influenza A and B infections during the 2012-2013 influenza season in Italy. *BMC Infect Dis* 2016; **16**: 6 [PMID: 26743673 DOI: 10.1186/s12879-015-1333-x]

3 **Ruf BR**, Knuf M. The burden of seasonal and pandemic influenza in infants and children. *Eur J Pediatr* 2014; **173**: 265-276 [PMID: 23661234 DOI: 10.1007/s00431-013-2023-6]

4 **Principi N**, Scala A, Daleno C, Esposito S. Influenza C virus–associated community-acquired pneumonia in children. *Influenza Other Respir Viruses* 2013; **7**: 999-1003 [PMID: 23594251 DOI: 10.1111/irv.12062]

5 **Tafalla M**, Buijssen M, Geets R, Vonk Noordegraaf-Schouten M. A comprehensive review of the epidemiology and disease burden of Influenza B in 9 European countries. *Hum Vaccin Immunother* 2016; **12**: 993-1002 [PMID: 26890005 DOI: 10.1080/21645515.2015.1111494]

6 **Yan S**, Weycker D, Sokolowski S. US healthcare costs attributable to type A and type B influenza. *Hum Vaccin Immunother* 2017; **13**: 2041-2047 [PMID: 28700268 DOI: 10.1080/21645515.2017.1345400]

7 **Zhu D**, Lok C, Chao S, Chen L, Li R, Zhao Z, Dong J, Qin K, Zhao X. Detection and characterization of type B influenza virus from influenza-like illness cases during the 2017-2018 winter influenza season in Beijing, China. *Arch Virol* 2019; **164**: 995-1003 [PMID: 30729995 DOI: 10.1007/s00705-019-04160-w]

8 **Dai Z**, Zhang L, Yu Q, Liu L, Yang M, Fan K. Early Administration of Oseltamivir Within 48 Hours After Onset of Flulike Symptoms Can Reduce the Risk of Influenza B Virus-Associated Pneumonia in Hospitalized Pediatric Patients with Influenza B Virus Infection. *Pediatr Infect Dis J* 2020; **39**: e20-e22 [PMID: 31929434 DOI: 10.1097/INF.0000000000002528]

9 **Heikkinen T**, Silvennoinen H, Peltola V, Ziegler T, Vainionpaa R, Vuorinen T, Kainulainen L, Puhakka T, Jartti T, Toikka P, Lehtinen P, Routi T, Juven T. Burden of influenza in children in the community. *J Infect Dis* 2004; **190**: 1369-1373 [PMID: 15378427 DOI: 10.1086/424527]

10 **Narayan VV**, Iuliano AD, Roguski K, Bhardwaj R, Chadha M, Saha S, Haldar P, Kumar R, Sreenivas V, Kant S, Bresee J, Jain S, Krishnan A. Burden of influenza-associated respiratory and circulatory mortality in India, 2010-2013. *J Glob Health* 2020; **10**: 010402 [PMID: 32373326 DOI: 10.7189/jogh.10.010402]

11 **Mosnier A**, Caini S, Daviaud I, Nauleau E, Bui TT, Debost E, Bedouret B, Agius G, van der Werf S, Lina B, Cohen JM; GROG network. Clinical Characteristics Are Similar across Type A and B Influenza Virus Infections. *PLoS One* 2015; **10**: e0136186 [PMID: 26325069 DOI: 10.1371/journal.pone.0136186]

12 **Kini S**, Bhat Y R, Handattu K, Kousika P, Thunga C. Spectrum of Influenza B Viral Infection in Indian Children: A Tertiary Centre Experience. *J Nepal Paediatr Soc* 2018; **38**: 170-175 [DOI: 10.3126/jnps.v38i3.23119]

13 **Peltola V**, Ziegler T, Ruuskanen O. Influenza A and B virus infections in children. *Clin Infect Dis* 2003; **36**: 299-305 [PMID: 12539071 DOI: 10.1086/345909]

14 **Caini S**, Kusznierz G, Garate VV, Wangchuk S, Thapa B, de Paula Júnior FJ, Ferreira de Almeida WA, Njouom R, Fasce RA, Bustos P, Feng L, Peng Z, Araya JL, Bruno A, de Mora D, Barahona de Gámez MJ, Pebody R, Zambon M, Higueros R, Rivera R, Kosasih H, Castrucci MR, Bella A, Kadjo HA, Daouda C, Makusheva A, Bessonova O, Chaves SS, Emukule GO, Heraud JM, Razanajatovo NH, Barakat A, El Falaki F, Meijer A, Donker GA, Huang QS, Wood T, Balmaseda A, Palekar R, Arévalo BM, Rodrigues AP, Guiomar R, Lee VJM, Ang LW, Cohen C, Treurnicht F, Mironenko A, Holubka O, Bresee J, Brammer L, Le MTQ, Hoang PVM, El Guerche-Séblain C, Paget J; Global Influenza B Study team. The epidemiological signature of influenza B virus and its B/Victoria and B/Yamagata lineages in the 21st century. *PLoS One* 2019; **14**: e0222381 [PMID: 31513690 DOI: 10.1371/journal.pone.0222381]

15 **Daley AJ**, Nallusamy R, Isaacs D. Comparison of influenza A and influenza B virus infection in hospitalized children. *J Paediatr Child Health* 2000; **36**: 332-335 [PMID: 10940165 DOI: 10.1046/j.1440-1754.2000.00533.x]

16 **Lennon DR**, Cherry JD, Morgenstein A, Champion JG, Bryson YJ. Longitudinal study of influenza B symptomatology and interferon production in children and college students. *Pediatr Infect Dis* 1983; **2**: 212-215 [PMID: 6191312 DOI: 10.1097/00006454-198305000-00008]

17 **Grant KA**, Carville K, Fielding JE, Barr IG, Riddell MA, Tran T, Kelly HA. High proportion of influenza B characterises the 2008 influenza season in Victoria. *Commun Dis Intell Q Rep* 2009; **33**: 328-336 [PMID: 20043604]

18 **Bedford T**, Riley S, Barr IG, Broor S, Chadha M, Cox NJ, Daniels RS, Gunasekaran CP, Hurt AC, Kelso A, Klimov A, Lewis NS, Li X, McCauley JW, Odagiri T, Potdar V, Rambaut A, Shu Y, Skepner E, Smith DJ, Suchard MA, Tashiro M, Wang D, Xu X, Lemey P, Russell CA. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. *Nature* 2015; **523**: 217-220 [PMID: 26053121 DOI: 10.1038/nature14460]

19 **Chi CY**, Wang SM, Lin CC, Wang HC, Wang JR, Su IJ, Liu CC. Clinical features of children infected with different strains of influenza B in southern Taiwan. *Pediatr Infect Dis J* 2008; **27**: 640-645 [PMID: 18520968 DOI: 10.1097/INF.0b013e31816be008]

20 **McCullers JA**, Facchini S, Chesney PJ, Webster RG. Influenza B virus encephalitis. *Clin Infect Dis* 1999; **28**: 898-900 [PMID: 10825057 DOI: 10.1086/515214]

21 **Straumanis JP**, Tapia MD, King JC. Influenza B infection associated with encephalitis: treatment with oseltamivir. *Pediatr Infect Dis J* 2002; **21**: 173-175 [PMID: 11840091 DOI: 10.1097/00006454-200202000-00021]

22 **Eşki A**, Öztürk GK, Gülen F, Çiçek C, Demir E. Risk Factors for Influenza Virus Related Severe Lower Respiratory Tract Infection in Children. *Pediatr Infect Dis J* 2019; **38**: 1090-1095 [PMID: 31469782 DOI: 10.1097/INF.0000000000002447]

23 **Vemula SV**, Zhao J, Liu J, Wang X, Biswas S, Hewlett I. Current Approaches for Diagnosis of Influenza Virus Infections in Humans. *Viruses* 2016; **8**: 96 [PMID: 27077877 DOI: 10.3390/v8040096]

24 **Gaitonde DY**, Moore FC, Morgan MK. Influenza: Diagnosis and Treatment. *Am Fam Physician* 2019; **100**: 751-758 [PMID: 31845781]

25 **Sheng Z**, Huang C, Liu R, Guo Y, Ran Z, Li F, Wang D. Next-Generation Sequencing Analysis of Cellular Response to Influenza B Virus Infection. *Viruses* 2020; **12** [PMID: 32244344 DOI: 10.3390/v12040383]

26 **Sato M**, Saito R, Sato I, Tanabe N, Shobugawa Y, Sasaki A, Li D, Suzuki Y, Sato M, Sakai T, Oguma T, Tsukada H, Gejyo F, Suzuki H. Effectiveness of oseltamivir treatment among children with influenza A or B virus infections during four successive winters in Niigata City, Japan. *Tohoku J Exp Med* 2008; **214**: 113-120 [PMID: 18285668 DOI: 10.1620/tjem.214.113]

27 **Suzuki E**, Ichihara K. The course of fever following influenza virus infection in children treated with oseltamivir. *J Med Virol* 2008; **80**: 1065-1071 [PMID: 18428131 DOI: 10.1002/jmv.21144]

28 **Hedrick JA**, Barzilai A, Behre U, Henderson FW, Hammond J, Reilly L, Keene O. Zanamivir for treatment of symptomatic influenza A and B infection in children five to twelve years of age: a randomized controlled trial. *Pediatr Infect Dis J* 2000; **19**: 410-417 [PMID: 10819336 DOI: 10.1097/00006454-200005000-00005]

29 **Whitley RJ**, Hayden FG, Reisinger KS, Young N, Dutkowski R, Ipe D, Mills RG, Ward P. Oral oseltamivir

treatment of influenza in children. *Pediatr Infect Dis J* 2001; **20**: 127-133 [PMID: 11224828 DOI: 10.1097/00006454-200102000-00002]

30 **Matheson NJ**, Symmonds-Abrahams M, Sheikh A, Shepperd S, Harnden A. Neuraminidase inhibitors for preventing and treating influenza in children. *Cochrane Database Syst Rev* 2003; **3**: CD002744 [PMID: 12917931 DOI: 10.1002/14651858.CD002744]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2020 November 19; 9(3): 53-62

DOI: 10.5409/wjcp.v9.i3.53

ISSN 2219-2808 (online)

ORIGINAL ARTICLE

Basic Study

Identification and characterization of resistance and pathogenicity of *Enterococcus spp.* in samples of donor breast milk

Luana Andrade Mendes Santana, Nívea Nara Novais Andrade, Lucas Santana Coelho da Silva, Caline Novais Teixeira Oliveira, Breno Bittencourt de Brito, Fabrício Freire de Melo, Cláudio Lima Souza, Lucas Miranda Marques, Márcio Vasconcelos Oliveira

ORCID number: Luana Andrade Mendes Santana 0000-0001-7527-3140; Nívea Nara Novais Andrade 0000-0001-9372-7548; Lucas Santana Coelho da Silva 0000-0003-0555-0696; Caline Novais Teixeira Oliveira 0000-0003-3094-4363; Breno Bittencourt de Brito 0000-0002-1831-7909; Fabrício Freire de Melo 0000-0002-5680-2753; Cláudio Lima Souza 0000-0002-8094-8357; Lucas Miranda Marques 0000-0002-8276-8149; Márcio Vasconcelos Oliveira 0000-0002-8959-0478.

Author contributions: Santana LAM, Andrade NNN, da Silva LSC, Oliveira CNT, de Brito BB, de Melo FF, Souza CL, Marques LM, and Oliveira MV contributed to the conception and design of the study, literature review and analysis, drafting and critical revision and editing. All authors contributed equally to approval of the final version.

Institutional review board statement: This study was reviewed and approved by the Ethical Committee of Research in Human Beings of the Multidisciplinary Institute of Health - Campus Anísio Teixeira from the Federal University of Bahia (IMS-CAT/ Universidade Federal da Bahia) under the

Luana Andrade Mendes Santana, Nívea Nara Novais Andrade, Caline Novais Teixeira Oliveira, Breno Bittencourt de Brito, Fabrício Freire de Melo, Cláudio Lima Souza, Lucas Miranda Marques, Márcio Vasconcelos Oliveira, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista 45029-094, Bahia, Brazil

Lucas Santana Coelho da Silva, Campus Soane Nazaré de Andrade, Universidade Estadual de Santa Cruz, Ilhéus 45662-900, Bahia, Brazil

Corresponding author: Fabrício Freire de Melo, MSc, PhD, Postdoc, Professor, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Rua Hormindo Barros, 58, Quadra 17, Lote 58, Vitória da Conquista 45029-094, Bahia, Brazil. freiremelo@yahoo.com.br

Abstract

BACKGROUND

Breast milk is the primary source of nutrition for newborns. Hospitalized babies frequently need nutritional support from Human Milk Banks. As bacterial species of the genus *Enterococcus* are part of the microbiota of healthy donors, they may contaminate samples of pumped breast milk.

AIM

To identify and characterize the bacterial virulence and resistance in samples isolated from the nipple-areolar region, hands, and breast milk aliquots from donors at the Human Milk Bank of Municipal Hospital Esaú Matos in the city of Vitória da Conquista, Bahia State, Brazil.

METHODS

The personal hygiene and sanitation of donors were analyzed with the aim of identifying possible reasons for contamination of pumped milk. Cutaneous samples as well as aliquots of unpasteurized and pasteurized milk from 30 participants were obtained. Each *Enterococcus spp.* isolate underwent a disk diffusion susceptibility test and molecular biology techniques to determine resistance and virulence genes.

RESULTS

Enterococcus spp. were identified in 30% of donors (n = 9), and 11 specimens were

protocol number 80800717.8.0000.5556 and committee opinion number 2.475.023.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

Data sharing statement: There are no additional data available.

Manuscript source: Unsolicited manuscript

Specialty type: Infectious diseases

Country/Territory of origin: Brazil

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

Received: August 17, 2020
Peer-review started: August 17, 2020
First decision: September 13, 2020
Revised: September 17, 2020
Accepted: September 25, 2020
Article in press: September 25, 2020
Published online: November 19, 2020

P-Reviewer: Berardi A, Liu H
S-Editor: Zhang L
L-Editor: Webster JR
P-Editor: Wu YXJ

isolated. Resistance to tetracycline was highly prevalent, being detectable in 63% of the isolates (n = 7) and followed by intermediate sensitivity to ciprofloxacin, observed in 27% of the specimens (n = 3). The *efa*A gene was found in 63% (n = 7) of the isolates, while the *ace* gene was detected in 27% (n = 3).

CONCLUSION

This study illustrates the importance of microbiological monitoring by Human Milk Banks and the need for alternatives to prevent the presence of *Enterococcus spp*. in hospital settings.

Key Words: *Enterococcus spp.*; Breast milk; Virulence; Human milk; Pasteurization; Antimicrobial resistance

Core Tip: Human milk is the primary source of nutrition for newborns. Hospitalized newborns frequently require nutritional support from a Human Milk Bank. *Enterococcus spp.* can contaminate human milk, as they are part of the microbiota of healthy donors. This microbial genus is important in human diseases, mainly among newborns; therefore, this study aimed to identify and characterize the virulence and resistance of *Enteroccocus spp.* isolated from samples from the nipple-areolar region, hands, and raw and pasteurized milk aliquots from donors at a Brazilian Human Milk Bank.

Citation: Santana LAM, Andrade NNN, da Silva LSC, Oliveira CNT, de Brito BB, de Melo FF, Souza CL, Marques LM, Oliveira MV. Identification and characterization of resistance and pathogenicity of *Enterococcus spp*. in samples of donor breast milk. *World J Clin Pediatr* 2020; 9(3): 53-62
URL: https://www.wjgnet.com/2219-2808/full/v9/i3/53.htm
DOI: https://dx.doi.org/10.5409/wjcp.v9.i3.53

INTRODUCTION

There is no ideal substitute for human milk (HM) during early life despite current technological advances in baby food production, processing, preservation, and preparation[1]. Unquestionably, HM supplies newborns (NBs) with all the nutrients that adequately meet the physiological and metabolic needs in this age group[2].

Premature births and hospitalizations in neonatal intensive care units as well as maternal illness, death, and low milk production can hinder the introduction and maintenance of breastfeeding. In this context, the use of donated HM has become an efficient alternative to breastfeeding[2] and allows NBs to be fed with milk obtained from Human Milk Banks (HMBs)[3]. This strategy involves the collection, processing, and quality control of colostrum, transition milk, and mature milk for later distribution to NBs under a doctor or nutritionist prescription[4].

Enterococcus species are associated with important infections in NBs and are potential contaminants of milked human milk (MHM) samples. *Enterococcus spp.* are part of the gastrointestinal tract microbiota in healthy humans[5] and may be present in significant numbers on the hands and utensils of milk donors. The presence of these bacteria in human milk indicates that they are due to direct or indirect contact with fecal material, compromising the quality of the MHM[6]. Some *Enterococcus spp.*, especially *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*), are highly pathogenic in NBs and must be effectively inactivated through pasteurization at HMBs.

To date, there are no published data on the isolation of enteric bacteria with hygienic-sanitary conditions of household collections performed by MHM donors. Considering the propensity of donated milk to be contaminated by these microorganisms, the clinical importance of such pathogens, and their ability to be dispersed through vectors such as health professionals and insects[7], it is important to investigate their virulence and dispersion capacity. Moreover, it is crucial to evaluate

the quality of the collection, preservation, and processing of the HM offered to NBs. These procedures are essential for a better understanding of the pathogenicity of *Enterococcus spp*. present in raw and pasteurized human milk samples and in samples taken from the hands and nipple-areolar region of donors linked to HMBs.

MATERIALS AND METHODS

Study design, period, region, and population

This is a cross-sectional study performed using samples from the nipple-areolar region, hands, and aliquots of raw and pasteurized MHM from healthy donors registered at the HMB of the Municipal Hospital Esaú Matos in the city of Vitória da Conquista, State of Bahia, Brazil.

The present study recruited donors registered at the above-mentioned HMB who had a donation history with a mean weekly volume of donated milk greater than or equal to 400 mL. Donated samples of raw milk with a volume less than 400 mL were pooled with milk samples from other donors for pasteurization, thus losing the traceability and specificity of the sample from a specific donor. The exclusion criteria were: donors with wounds on the hand or nipple-areolar region and donation of raw milk samples with a milk volume lower than that required for individual pasteurization.

The microbial isolates analyzed in the present study were obtained by the single collection of samples from the nipple-areolar region and hands as well as aliquots of MHM before and after pasteurization from 30 donors between March and August 2018. The collections were carried out at the donors' residences during scheduled visits. In addition to microbiological evaluation of the above-mentioned biological samples, questionnaires on hygienic-sanitary issues regarding the collection, packaging, and preservation of the donated milk were completed by the participants.

Ethical considerations

The study was approved by the Ethics Committee on Research with Human Beings of the Multidisciplinary Institute of Health of the Universidade Federal da Bahia, under the Certificate of Presentation for Ethics Apprehension number 80800717.8.0000.5556 and committee opinion number 2.475.023. The study followed all the conditions established by the Resolution 466/2012 of the Brazilian National Health Council.

Isolation, identification, evaluation of antimicrobial activity, and detection of constitutive, virulence, and resistance genes in Enterococcus spp.

After collection, the biological samples were immediately inoculated into enrichment brain heart infusion broth (HIMEDIA®) and incubated at 35-37ºC for 24 h. After that, they were placed in Petri dishes with 5% Blood Agar Medium (Base Agar, HIMEDIA®) and Chromagar Orientation (BD™ CHROMagar™ Orientation) in a bacteriological oven at 35-37ºC for 24 h. Both methods were important for increasing the isolation and identification of microorganisms of interest in the present study.

The colonies with presumptive characteristics of *Enterococcus spp*. selected in both media underwent Gram staining to evaluate the morphological characteristics as well as to perform the catalase test and subsequent tests to identify the genus *Enterococcus*, namely: hydrolysis of esculin in the presence of bile and growth in sodium chloride (NaCl) 6.5% media.

All *Enterococcus spp*. isolates underwent evaluation of antimicrobial activity using the agar diffusion method[8-10], according to the recommendations of the Clinical and Laboratory Standards Institute (2017)[11].

With the aim of detecting the constitutive[12], virulence[13-16], and resistance[17,18] genes in *Enterococcus spp*., all samples that were positive in the identification step underwent polymerase chain reaction (PCR) after reactivation in brain heart infusion medium.

Statistical analysis

A descriptive analysis of the study variables was carried out from a database constituted in Microsoft Excel through the Statistical Program EPI INFO (version 7.2.2.6). For the frequency comparison, the chi-square test was used, with values of $P < 0.05$ (95% confidence interval) considered statistically significant.

RESULTS

Milk samples were collected from 30 donors registered at the HMB of the Municipal Hospital Esaú Matos, in Vitória da Conquista, Bahia State, Brazil. A total of 81 samples were collected: 30 samples from swabs containing skin samples from the nipple-areolar region and donors' hands, 30 samples of raw MHM, and 21 samples of pasteurized MHM. Following the methodological criteria, it was not possible to obtain an appropriate aliquot of pasteurized milk from 9 (30%) participants as they did not donate enough milk volume to perform pasteurization in a single bottle.

Enterococcus spp. strains were isolated from 9 donors (30%), and a total of 11 strains were identified: 7 strains (63.6%) from skin samples and 4 strains (36.4%) from raw MHM samples. Although *Enterococcus spp*. were not detected in the pasteurized MHM samples, in 38% (n = 8) of these samples other microorganisms, mainly *Staphylococcus aureus*, were isolated.

The study participants were questioned about socioeconomic aspects and hygienic-sanitary conditions in order to link selected variables to the route of human milk contamination by *Enterococcus spp*. In this context, most participants were aged 30 years or less, were married or living with a partner, had a family income above two minimum wages, had one child, and were educated to high school level.

With regard to the hygienic-sanitary procedures performed by the donors for milk collection, most participants reported that they always performed the following procedures: hand washing before the collection of milk (96%); held back hair before milking (80%); breast washing before milking (86.7%); used a mask while milking (86.7%); avoided talking during milking (60%); discarded the first jet of milk (53.3%); milk obtained using a manual/electric pump (80%). However, only 12 (80%) participants declared that they checked the size of their nails and trimmed them when necessary before milking (Table 1).

Socioeconomic variables of HM donors such as age (above or below 30 years), marital status (single or living as a couple), family income (higher or lower than 2 minimum wages), number of children (one or more than one), and educational level (high school or graduate) as well as variables related to hygienic-sanitary procedures including hand washing before milking, talking during milking, and discarding the first milk jet at the beginning of collection were not significantly associated with the detection of *Enterococcus spp*. in samples ($P < 0.05$) (Table 1).

Among the 11 isolates of *Enterococcus spp*., antimicrobial resistance was found in 10 isolates (91%). Resistance to tetracycline was most common (63% of isolates, n = 7), and intermediate sensitivity to ciprofloxacin was found in 27% of the isolates (n = 3). Resistance to quinolones (ciprofloxacin, norfloxacin, and levofloxacin) was observed in 9% of the isolates (n = 1) (Table 2). All isolates were sensitive to vancomycin. All specimens were analyzed by PCR to detect quinolone resistance genes (*gyr*A and *par*C genes), but no positive results were obtained.

The *Enterococcus spp*. isolates were analyzed by PCR for detection of the constitutive *ddl* gene encoding the D-alanine ligases, which are species-specific for *E. faecalis* and *E. faecium*. This analysis showed that among the 11 enterococcal strains from 9 participants, 6 isolates were identified as *Enterococcus spp*. and 5 isolates were identified as *E. faecalis*. However, three *E. faecalis* isolates were found to be simultaneously colonized by *E. faecium* strains. Thus, three donors had *E. faecalis* and *E. faecium* strains. Such detection was possible because, after the initial identification of the *Enterococcus* genus, the stored colonies were collected from the CHROMagar Orientation medium (BD™ CHROMagar™ Orientation), which identified the genus and not the species of *Enterococcus*; thus, colonies of both species were collected.

All isolates of *Enterococcus spp*. underwent PCR for detection of the virulence genes *ace*, *efa*A, *gel*E, *as*, *cyl*A, *hyl*, *esp*, *as*, *cyl*A, *hyl*, and *esp* (Table 3). Among the 11 isolates, 7 isolates (63%) had the *efa*A gene that encodes the surface antigen A, which is commonly associated with biofilm formation and endocarditis, and 3 isolates (27%) had the *ace* gene, which encodes the protein that adheres to collagen and laminin, favoring bacterial adhesion commonly associated with endocarditis. The following enterocin genes were also genotypically investigated by PCR in *Enterococcus spp*.: Enterocin A, Enterocin B, Enterocin P, and Enterocin 31, also known as Bacteriocin. However, none of the isolates were positive for these genes.

DISCUSSION

Enterococcus spp. are present in the human microbiota and are associated with

Table 1 Descriptive analysis of the socioeconomic aspects and hygienic-sanitary conditions related to the collection of human milk and to human milk donors (*n* = 30) registered in the Human Milk Bank of the Municipal Hospital Esaú Matos in Vitória da Conquista, Bahia State, Brazil

Descriptive analysis of socioeconomic aspects of HM donors		
Variable	***n***	**(%)**
Age group		
Age less than 30 yr	19	63.3
Over 30 yr of age	11	36.7
Marital status/Conjugal state		
Married/living with spouse	20	66.7
Single or divorced/without a partner	10	33.3
Family income		
Less than 2 minimum wages[1]	9	30.0
Above 2 minimum wages	21	70.0
Number of children		
One child	16	53.3
Two or more children	14	36.7
Schooling		
High school	21	70.0
Graduate	9	30.0
Total	30	100.0
Descriptive analysis of hygienic-sanitary conditions of HM donors		
Variable	*n*	(%)
Hand washing prior to collection		
Sometimes/never	1	3.3
Always	29	96.7
Nail size check/trim before collection		
Sometimes/never	18	60.0
Always	12	40.0
Hair holding habit before collection		
Sometimes/never	6	20.0
Always	24	80.0
Breast lavage before collection		
Sometimes/never	4	13.3
Always	26	86.7
Use of mask during collection		
Sometimes/never	4	13.3
Always	26	86.7
Avoid talking during collection		
Sometimes/never	12	40.0
Always	18	60.0
Discard first jet of milk at the beginning of collection		
Sometimes/never	14	46.7

Always	16	53.3
Milk collection		
Manual/electric pump	24	80.0
Manual milking	6	20.0
Cleaning frequency of the pump[2]		
Sometimes/never	0	0
Always before using	24[1]	100.0[1]
Total	30	100.0

[1]Minimum wage in the period: BRL954,00. [2]Question applied only to donors who reported performing milking by hand and using an electric pump.

Table 2 Sensitivity profile of the isolates (*n* = 11) of *Enterococcus spp.* found in relation to the list of antimicrobials evaluated following the recommendations of the Clinical and Laboratory Standards Institute (2017)

Antimicrobials	Sensitive, *n* (%)	Intermediate, *n* (%)	Resistant, *n* (%)
Ampicillin 10 μg	11 (100.0)	0 (0)	0 (0)
Penicillin 10 un	9 (81.8)	0 (0)	2 (18.2)
Tetracycline 30 μg	4 (36.4)	0 (0)	7 (63.6)
Ciprofloxacin 5 μg	7 (63.6)	3 (27.3)	1 (9.1)
Norfloxacin 10 μg	9 (81.8)	0 (0%)	2 (18.2)
Levofloxacin 5 μg	10 (90.9)	0 (0)	1 (9.1)
Nitrofurantoin 300 μg	9 (81.8)	0 (0)	2 (18.2)
Linezolid 30 μg	11 (100.0)	0 (0)	0 (0)
Vancomycin 30 μg	11 (100.0)	0 (0)	0 (0)

un: Units.

Table 3 Distribution of the virulence genes of *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus spp.*

Virulence genes	*E. faecalis*		*E. faecium*		*Enterococcus spp.*		Total	
	N (isolated)	%	*N* (isolated)	%	*N* (isolated)	%	*N* (isolated)	%
Ace	1[a]	33	1[a]	33	2	67	3	27
*efa*A	3[b]	43	2[b]	28	3	43	7	63
*gel*E	0	-	0	-	0	-	0	-
As	0	-	0	-	0	-	0	-
*cyl*A	0	-	0	-	0	-	0	-
Hyl	0	-	0	-	0	-	0	-
Esp	0	-	0	-	0	-	0	-

[a]*ace* gene found in 1 isolate (containing strains of *Enterococcus faecalis* and *Enterococcus faecium*). [b]*efa*A gene found in three *Enterococcus faecalis* isolates (being a single isolate of *Enterococcus faecalis* and two isolates of *Enterococcus faecalis* and *Enterococcus faecium*). *E. faecalis: Enterococcus faecalis*; *E. faecium: Enterococcus faecium*.

important infections in NBs, and are potential contaminants of HM samples[8]. To date, there are no studies correlating NB infections and infant colonization with *Enterococcus spp*. in the city where this study was carried out. Moreover, there are no data associating socioeconomic aspects and hygienic-sanitary conditions regarding HM donors with the isolation of enteric bacteria. In the present study, *Enterococcus spp.*

were isolated from 30% of the participants. Similarly, *Enterococcus ssp*. were isolated from raw MHM samples within four[19] and seven[20] days after delivery. In such studies, gram-positive bacteria, including *E. faecalis*[21], *E. faecium*[21,22], *Staphylococcus spp*[23] and fecal coliforms[2] were identified in MHM[19], but they were not detected after milk pasteurization; however, other microorganisms continued to be detected after pasteurization.

Similar findings were observed in other HMBs in which the isolation rates were 2%[2], 6%[2], 7.5%[21], and even 70.4%[3] in non-pasteurized MHM samples and was 50.7% in pasteurized MHM[3] samples. These data reinforce the importance of strict hygienic-sanitary procedures in HMBs as well as the microbiological control of HM samples.

Among the 11 *Enterococcus spp*. isolated in this study, different types of resistance to antibiotics were found in 91% of the isolates (n = 10). In the phenotypical analysis of antimicrobial resistance, most isolates were susceptible to ampicillin, linezolid, and vancomycin, but resistance to tetracycline (63%), ciprofloxacin (27%), norfloxacin, levofloxacin, nitrofurantoin (9%), and penicillin was detected. *Enterococcus spp*. resistance has been previously reported in samples from miscellaneous clinical materials[22], food, and clinical samples[23], with resistance rates of 5% to tetracycline[22] and of 3%[22] and 70.5%[23] to ciprofloxacin.

Despite the identification of quinolone resistance, the resistance genes *gyr*A and *par* C were not detected. This phenomenon may be associated with interferences, including differences in the pH of the Mueller Hinton media, in mean temperature, and in the density of the inoculum applied. Due to *Enterococcus spp*. resistance to antimicrobials such as penicillin, ampicillin, aminoglycosides, tetracycline, and vancomycin and its association with nosocomial infections[24], it is crucial to identify these bacteria in different types of biological samples, including HM.

In this study, the presence of virulence factors in the isolated strains was also evaluated, and the genes *efa*A and *ace* were found in 63% and 27% of the isolates, respectively. These genes are associated with enterococcal biofilm formation and endocarditis, making the treatment of infections caused by these microorganisms difficult. With regard to species identification, of the three isolates with the *ace* gene, two were *Enteroccocus spp*. and one had *E. faecium* and *E. faecalis* strains. Among the seven isolates carrying the *efa*A gene, three were *Enterococcus spp*., two were *E. faecalis*, and two had *E. faecalis* and *E. faecium* strains.

The genes *ace* and *efa*A have been previously detected in *E. faecalis* and *E. faecium*[22] hospital strains and the *ace* gene has also been found in *Enterococcus spp*. of clinical origin[23]. The pathogenicity mechanisms of *Enterococcus spp*. are not yet fully understood. It is known that these microorganisms have virulence factors that are associated with the severity and duration of human infections as well as with the dissemination of these microorganisms in the hospital environment, where they mainly cause urinary infections, endocarditis, and bacteremia. The most frequently reported virulence factors are related to the adhesion to host tissues, invasion, abscess formation, modulation of the host inflammatory responses, and secretion of toxic products[25].

The high percentage of *Enterococcus spp*. isolates in HM samples associated with the high antimicrobial resistance rate (mainly to tetracycline and ciprofloxacin) and the positivity of virulence factors (detection of the genes *ace* and *efa*A) found in this study confirm the importance of carrying out strict control from the collection to the distribution of MHM. In this context, it is necessary to take utmost care during milk processing in order to avoid the dispersion of microorganisms, as the entry of these microorganisms into the hospital environment can increase the risk of infection in hospitalized patients, mainly in those with a weakened immune system.

CONCLUSION

The present study demonstrates the necessity for microbiological control in HMBs and the requirement to develop alternatives to avoid the entry of *Enterococcus spp*. species into the hospital environment, which can potentially lead to important NB infections that increase the length of hospitalizations and, consequently, health service costs. Considering that most premature NBs are transferred to intensive care units, thereby requiring food supplementation with MHM, and that the consumption of contaminated MHM can lead to mild-to-severe infections, the accurate identification of bacterial pathogens is an essential requirement for the detection of microorganism reservoirs, infection sources, HM distribution monitoring, and decision taking aiming to reduce health risks in NBs. Moreover, strict control from the collection to the

distribution of donated HM may reduce the dispersion rates of virulent and multidrug-resistant microorganisms that can cause several problems in a hospital environment, leading to increases in morbidity, mortality, and costs related to health care.

ARTICLE HIGHLIGHTS

Research background

Human milk is the primary source of nutrition for newborns. Hospitalized babies often require nutritional support from Human Milk Banks. As *Enterococcus spp*. are part of the microbiota of healthy donors, they are potential contaminants of pumped breast milk.

Research motivation

Donor Human Milk is an important alternative that allows the appropriate feeding of hospitalized newborns. A number of steps are needed to ensure the safe collection, processing, and quality control of colostrum, transition milk, and mature milk for subsequent distribution to newborns under a doctor or nutritionist prescription. However, such milk can be contaminated by *Enterococcus spp*. species that are highly pathogenic in newborns, and can increase morbidity, hospitalization time, and mortality among neonates.

Research objectives

The present study intended to identify and describe the bacterial virulence and resistance in samples obtained from the nipple-areolar region, hands, and breast milk aliquots of donors at the HMB of Municipal Hospital Esaú Matos in the city of Vitória da Conquista, Bahia State, Brazil.

Research methods

This cross-sectional study used samples from the nipple-areolar region, hands, and aliquots of raw and pasteurized milked human milk from healthy human milk donors. The study recruited donors who had a donation history of a mean weekly volume of donated milk equal to or higher than 400 mL. The microbial isolates from milk samples were analyzed before and after pasteurization. A total of 30 donors were assessed between March and August 2018.

The samples were immediately inoculated into enrichment brain heart infusion broth (HIMEDIA®) and incubated at 35-37°C for 24 h after collection. Thereafter, they were placed in Petri dishes with 5% Blood Agar Medium (Base Agar, HIMEDIA®) and Chromagar Orientation (BD™ CHROMagar™ Orientation) in an incubator at 35-37°C for 24 h.

Colonies in which *Enterococcus spp*. were observed were selected from both media and underwent Gram staining to evaluate the morphological characteristics and to perform the catalase test and subsequent tests to identify the genus *Enterococcus*.

All *Enterococcus spp*. isolates underwent evaluation of antimicrobial activity by the agar diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute (2017).

To detect the constitutive, virulence, and resistance genes in *Enterococcus spp*., all samples that were positive in the identification step underwent polymerase chain reaction after reactivation in brain heart infusion media.

A descriptive analysis of the study variables was carried out from a database constituted in Microsoft Excel through the Statistical Program EPI INFO (version 7.2.2.6). For the frequency comparison, the chi-square test was used, with values of $P < 0.05$ (95% confidence interval) considered statistically significant.

Research results

Eighty-one samples were collected, with 30 samples from swabs containing nipple-areolar region skin samples and donors' hands, 30 samples of raw milked human milk, and 21 samples of pasteurized milked human milk. *Enterococcus spp*. strains were isolated from 30% of the donors and 11 strains were identified: 7 strains (63.6%) from skin samples and 4 strains (36.4%) from raw MHM samples.

The majority of participants declared that they washed their hands before the collection of milk (96%), held back hair before milking (80%, washed the breasts before milking (86.7%), used a mask while milking (86.7%), avoided talking during milking

(60%), discarded the first jet of milk (53.3%), and obtained milk using a manual/electric pump (80%).

No statistically significant associations between demographic and hygienic characteristics and the detection of *Enterococcus spp*. were observed. Antimicrobial resistance was identified in 10 isolates (91%), mainly to tetracycline.

Research conclusions

Here we demonstrate the importance of appropriate microbiological control in Human Milk Banks in order to prevent *Enterococcus spp*. in hospitals as these bacteria can lead to important newborn infections that increase morbidity, mortality, and hospitalizations among these patients, and increase health service costs.

Research perspectives

This study adds information to the microbiological control of donated milk, which, if well-performed from human milk collection to its distribution, may reduce the dispersion rates of virulent and multidrug-resistant microorganisms in a hospital environment, avoiding unwanted clinical outcomes among newborns. Putting such measures into practice may improve the health of children around the world.

REFERENCES

1 **Silva VAAL**, Caminha MFC, Silva SL, Serva VMSBD, Azevedo PTACC, Filho MB. Maternal breastfeeding: indicators and factors associated with exclusive breastfeeding in a subnormal urban cluster assisted by the Family Health Strategy. *J Pediatr (Rio J)* 2019; **95**: 298-305 [PMID: 29530663 DOI: 10.1016/j.jped.2018.01.004]

2 **Borges MS**, Oliveira AMM, Hattori WT, Abdallah VOS. Quality of human milk expressed in a human milk bank and at home. *J Pediatr (Rio J)* 2018; **94**: 399-403 [PMID: 28859913 DOI: 10.1016/j.jped.2017.07.004]

3 **Serafini AB**, André MC, Rodrigues MA, Kipnis A, Carvalho CO, Campos MR, Monteiro EC, Martins F, Jubé TF. [Microbiological quality of human milk from a Brazilian milk bank]. *Rev Saude Publica* 2003; **37**: 775-779 [PMID: 14666308 DOI: 10.1590/s0034-89102003000600013]

4 **de Almeida JA**, Novak FR. [Breastfeeding: a nature-culture hybrid]. *J Pediatr (Rio J)* 2004; **80**: S119-S125 [PMID: 15583761 DOI: 10.2223/1242]

5 **Murray PR**. Medical Microbiology. 4th ed. Guanabara Koogan, 2004: 220-223

6 **Novak FR**, Almeida JA, Asensi MD, Moraes BA, dos Prazeres Rodrigues D. [Antimicrobial resistance of coliform isolates from expressed human milk]. *Cad Saude Publica* 2001; **17**: 713-717 [PMID: 11395808 DOI: 10.1590/s0102-311x2001000300026]

7 **Oliveira PS**, Souza SG, Campos GB, da Silva DC, Sousa DS, Araújo SP, Ferreira LP, Santos VM, Amorim AT, Santos AM, Timenetsky J, Cruz MP, Yatsuda R, Marques LM. Isolation, pathogenicity and disinfection of Staphylococcus aureus carried by insects in two public hospitals of Vitória da Conquista, Bahia, Brazil. *Braz J Infect Dis* 2014; **18**: 129-136 [PMID: 24216155 DOI: 10.1016/j.bjid.2013.06.008]

8 **RAZIL**. National Health Surveillance Agency. Brazilian Pharmacopoeia. 5th ed. Anvisa: 2010: 59-73

9 **Fracalanzza SA**, Scheidegger EM, Santos PF, Leite PC, Teixeira LM. Antimicrobial resistance profiles of enterococci isolated from poultry meat and pasteurized milk in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 2007; **102**: 853-859 [PMID: 18060316 DOI: 10.1590/s0074-02762007005000120]

10 **Valenzuela AS**, Benomar N, Abriouel H, Cañamero MM, Gálvez A. Isolation and identification of Enterococcus faecium from seafoods: antimicrobial resistance and production of bacteriocin-like substances. *Food Microbiol* 2010; **27**: 955-961 [PMID: 20688238 DOI: 10.1016/j.fm.2010.05.033]

11 **CLSI**. M100-S26: Performance Standards for Antimicrobial Suscetibility Testing. 27th ed. Clinical and Laboratory Standards Institute, 2017

12 **Dutka-Malen S**, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995; **33**: 24-27 [PMID: 7699051 DOI: 10.1128/JCM.33.1.24-27.1995]

13 **Mannu L**, Paba A, Daga E, Comunian R, Zanetti S, Duprè I, Sechi LA. Comparison of the incidence of virulence determinants and antibiotic resistance between Enterococcus faecium strains of dairy, animal and clinical origin. *Int J Food Microbiol* 2003; **88**: 291-304 [PMID: 14597001 DOI: 10.1016/s0168-1605(03)00191-0]

14 **Vankerckhoven V**, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H. Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of Enterococcus faecium. *J Clin Microbiol* 2004; **42**: 4473-4479 [PMID: 15472296 DOI: 10.1128/JCM.42.10.4473-4479.2004]

15 **Shankar V**, Baghdayan AS, Huycke MM, Lindahl G, Gilmore MS. Infection-derived Enterococcus faecalis strains are enriched in esp, a gene encoding a novel surface protein. *Infect Immun* 1999; **67**: 193-200 [PMID: 9864215 DOI: 10.1128/IAI.67.1.193-200.1999]

16 **Vancanneyt M**, Lombardi A, Andrighetto C, Knijff E, Torriani S, Björkroth KJ, Franz CM, Foulquié Moreno MR, Revets H, De Vuyst L, Swings J, Kersters K, Dellaglio F, Holzapfel WH. Intraspecies genomic groups in Enterococcus faecium and their correlation with origin and pathogenicity. *Appl Environ Microbiol* 2002; **68**: 1381-1391 [PMID: 11872491 DOI: 10.1128/aem.68.3.1381-1391.2002]

17 **el Amin NA**, Jalal S, Wretlind B. Alterations in GyrA and ParC associated with fluoroquinolone resistance in Enterococcus faecium. *Antimicrob Agents Chemother* 1999; **43**: 947-949 [PMID: 10103206 DOI:

10.1128/AAC.43.4.947]

18 **Brisse S**, Fluit AC, Wagner U, Heisig P, Milatovic D, Verhoef J, Scheuring S, Köhrer K, Schmitz FJ. Association of alterations in ParC and GyrA proteins with resistance of clinical isolates of Enterococcus faecium to nine different fluoroquinolones. *Antimicrob Agents Chemother* 1999; **43**: 2513-2516 [PMID: 10508034 DOI: 10.1128/AAC.43.10.2513]

19 **Martín R**, Langa S, Reviriego C, Jimínez E, Marín ML, Xaus J, Fernández L, Rodríguez JM. Human milk is a source of lactic acid bacteria for the infant gut. *J Pediatr* 2003; **143**: 754-758 [PMID: 14657823 DOI: 10.1016/j.jpeds.2003.09.028]

20 **Martín R**, Heilig HG, Zoetendal EG, Jiménez E, Fernández L, Smidt H, Rodríguez JM. Cultivation-independent assessment of the bacterial diversity of breast milk among healthy women. *Res Microbiol* 2007; **158**: 31-37 [PMID: 17224259 DOI: 10.1016/j.resmic.2006.11.004]

21 **Silveira LAM**, D'Amorim MFG, Silva VRD, Terra APS. Controle Microbiológico do Leite Humano de um Hospital Universitário. *Revista Baiana de Saúde Pública* 2012; **36**: 844 [DOI: 10.22278/2318-2660.2012.v36.n3.a560]

22 **Kang M**, Xie Y, He C, Chen ZX, Guo L, Yang Q, Liu JY, Du Y, Ou QS, Wang LL. Molecular characteristics of vancomycin-resistant Enterococcus faecium from a tertiary care hospital in Chengdu, China: molecular characteristics of VRE in China. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 933-939 [PMID: 24463723 DOI: 10.1007/s10096-013-2029-z]

23 **Gomes BC**, Esteves CT, Palazzo IC, Darini AL, Felis GE, Sechi LA, Franco BD, De Martinis EC. Prevalence and characterization of Enterococcus spp. isolated from Brazilian foods. *Food Microbiol* 2008; **25**: 668-675 [PMID: 18541165 DOI: 10.1016/j.fm.2008.03.008]

24 **Hörner R**, Liscano MGM, Maraschin MM, Salla A, Meneghetti B, Nara L. Frasson Dal Forno NLFD, Righi RA. Suscetibilidade antimicrobiana entre amostras de Enterococcus isoladas no Hospital UniversitÃ¡rio de Santa Maria. *J Bras Patol Med Lab* 2005; **41**: 391-395 [DOI: 10.1590/S1676-24442005000600004]

25 **Eaton TJ**, Gasson MJ. Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. *Appl Environ Microbiol* 2001; **67**: 1628-1635 [PMID: 11282615 DOI: 10.1128/AEM.67.4.1628-1635.2001]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com