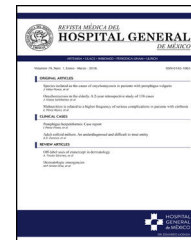




REVISTA MÉDICA DEL
HOSPITAL GENERAL
DE MÉXICO

www.elsevier.es/hgmx



ORIGINAL ARTICLE

Detection of antibiotic resistance genes β -lactamics in bacterial strains isolated from Umbilical Cord Blood Units for transplant



J.M. Bello-López*, J. Rojo-Medina

Centro Nacional de la Transfusión Sanguínea, Mexico City, Mexico

Received 15 January 2016; accepted 12 May 2016

Available online 30 May 2016

KEYWORDS

Antimicrobial resistance;
Antibiotics;
Hematopoietic stem cells;
Transplant;
Microbial contamination

Abstract

Introduction: Among the risks associated with hematopoietic stem cells transplantation of umbilical cord blood are bacterial infections. This becomes dangerous when bacterial sepsis is related to resistance to antibiotics commonly used to eliminate of this type infections. Among the widely used antibiotics are those of the β -lactam family. Among the genes responsible for the resistance are designated “*bla* genes” which encode for broad-spectrum lactamases. Its detection in the laboratory shown the importance in the manufacture of cord blood units.

Objective: To correlate the antibiotic resistance in bacterial contaminants isolated from Umbilical Cord Blood Units (UCBU), with detection of genes encoding resistance of β -lactamics antibiotics.

Materials and methods: Test of resistance to antibiotics was determined in 107 bacterial strains using disk diffusion method according to the recommendations of “The Clinical and Laboratory Standards Institute (CLSI)” and multiplex amplification by PCR of genes *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} were performed in order to detect genetic elements associated to antimicrobial resistance tested.

Results: Broad-spectrum penicillins showed lower inhibitory activity on the tested strains. Gene detection by multiplex PCR of encoding antimicrobial resistance (β -lactamases) revealed the presence of *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{TEM} genes in most strains tested even when these genes have been reported in *Enterobacteriaceae* family members.

Conclusions: The genotype detection encoding antibiotic resistance in isolates of UCBU clearly indicates the potential risk of sepsis difficult to eradicate in the patient, if present in a UCBU

* Corresponding author at: Centro Nacional de la Transfusión Sanguínea, Av. Othón de Mendizábal 195, Zacatenco, Gustavo A. Madero, Mexico City, Mexico. Telephone: +52 63922250x51658.

E-mail address: juanmanuelbello81@hotmail.com (J.M. Bello-López).

PALABRAS CLAVE

Resistencia
antimicrobiana;
Antibióticos;
Células troncales
hematopoyéticas;
Trasplante;
Contaminación
Microbiana

available for transplant. Additionally, the existence of these genes reveals horizontal transfer events of genetic material between bacteria. So the importance to realize this studies before transplant.

© 2016 Sociedad Médica del Hospital General de México. Published by Masson Doyma México S.A. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Detección de genes de resistencia a antibióticos β -lactámicos en cepas bacterianas aisladas de Unidades de Sangre de Cordón Umbilical para trasplante

Resumen

Introducción: Entre los riesgos asociados con el trasplante de células progenitoras hematopoyéticas de sangre de cordón umbilical son las infecciones bacterianas. Esto se convierte en peligroso cuando la sepsis está relacionada con la resistencia a antibióticos comúnmente utilizados para combatir infecciones. Entre los antibióticos ampliamente usados son los de la familia β -lactámicos. Los genes responsables de la resistencia a estos antibióticos se designan "genes *bla*" codificantes para lactamasas de amplio espectro. Su detección en el laboratorio demuestra la importancia del control en la manufactura de unidades de sangre de cordón.

Objetivo: Correlacionar la resistencia a los antibióticos en bacterias contaminantes aisladas de Unidades de Sangre de Cordón Umbilical (USCU), con la detección de genes codificantes a antibióticos β -lactámicos.

Materiales y métodos: Se realizaron ensayos de resistencia a antibióticos en 107 cepas por el método de difusión en disco de acuerdo a las recomendaciones de "El Instituto de Estándares Clínicos y de Laboratorio (CLSI)" y amplificación por PCR multiplex de genes *bla*_{TEM}, *bla*_{SHV} y *bla*_{CTX-M}.

Resultados: Las penicilinas de amplio espectro mostraron menor actividad inhibitoria en las cepas probadas. La detección de genes que codifican resistencia a antibióticos mediada por β -lactamasas reveló la presencia de variantes *bla*_{TEM}, *bla*_{SHV} y *bla*_{CTX-M} en la mayoría de las cepas, incluso cuando estos han sido reportados en miembros de la familia *Enterobacteriaceae*.

Conclusiones: La detección del genotipo de resistencia a antibióticos en aislamientos de USCU indica claramente el riesgo potencial de sepsis de difícil erradicación en el paciente, si estuviesen presentes en USCU disponibles para trasplante. La existencia de genes *bla* revela eventos de transferencia horizontal de ADN entre bacterias. De allí la importancia de realizar estos estudios antes del trasplante.

© 2016 Sociedad Médica del Hospital General de México. Publicado por Masson Doyma México S.A. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Transplantation of Umbilical Cord Blood (UCB) is one of the major technologies used to treat oncohematologic and non-oncohematologic disorders, since it is an invaluable source of Hematopoietic Stem Cells (HSC).^{1,2} The HSC transplantation has been used to treat diseases such as myeloblastic and lymphoblastic leukemia, primary immunodeficiencies, aplastic anemia, severe combined immunodeficiency, genetic and/or metabolic inherited diseases as mucopolysaccharidosis and leukodystrophies.³⁻⁵ However, the use and success of this type of transplantation is limited by several obstacles, mainly related to the donor-recipient compatibility, therefore the Human Leukocyte Antigen (HLA) typing becomes very important.⁶ It is well known that antigens of HLA Class I and Class II are the major immunological barrier to perform transplantation of

HSC,⁷ therefore patients included in transplant protocols are submitted to immunosuppression processes in order to prevent graft rejection; a dangerous situation for the patient who is exposed to a broad range of opportunistic infections by bacteria that normally would be considered innocuous.^{8,9} The procedure of collecting UCB must be performed under conditions that ensure its complete sterility. The risk of bacterial contamination is present in any stage, beginning with the collection, processing and cryopreservation.^{10,11} Therefore, in Cord Blood Banks (CBB) that collect and process UCB, the use of aerobic and anaerobic microbiological controls (preferably automated) is mandatory at the end of the process in order to ensure the safety of the final product. Antimicrobial treatment in immunocompromised patients is essential for the recovery after transplantation of HSC. In case of bacterial contamination of the UCBU, there is a potential risk of sepsis when

bacteria are resistant to antibiotics, making it difficult to eradicate with antimicrobial therapy. The presence of mobile genetic elements such as plasmids and transposons are associated directly with the acquisition of resistance to antibiotics in the nature.¹² A predominance of negative and Gram-positive bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis* and non-fermentative bacilli such as *Pseudomonas aeruginosa* have been recognized as contaminants,¹⁰ and producers of β -lactamases, generating resistance to penicillins, cephalosporins and monobactams.^{13,14} Antibiotics of the β -lactamic family have been widely used in the treatment of bacterial infections. Due to the irrational and indiscriminate use of antibiotics in nosocomial institutions, selection pressure results in emergence of multiresistant strains.¹⁵ Strains producing β -lactamases are responsible of nosocomial outbreaks, especially in intensive care units, complicating treatment and increasing morbidity and mortality.¹⁶ In a previous study, bacterial contaminants isolated from UCBU of CBB from National Center of Blood Transfusion for transplant were identified at molecular level; additionally the profile of antibiotic resistance and virulence factors were detected.¹⁷ However, additional tests were not performed, in order to know the genetic background of these strains and knowledge their possible acquisition of resistance genes. The aim of this work was research a correlation between pattern antibiotic resistances in bacterial strains isolated UCBU for transplant and the detection of genes coding for antibiotic resistance using Polymerase Chain Reaction (PCR) directed against β -lactamics genes.

Material and methods

Origin of the bacterial strains

The bacterial strains used in this study are listed in Table 1. All strains were isolated between 2003 and 2013 from discharged UCBU of the NCBT in Mexico City.¹⁷ 16S rRNA gene was amplified in all strains using the universal primers (27F and R1492) for bacteria and was sequenced. Additionally, ERIC-PCR assays to differentiate between unique and clones were done. All strains grew in Luria-Bertani (LB) broth (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl), or LB agar (supplemented with 1.5% agar).

Table 1 Strains used in this study.

Strain	Number of strains
<i>Enterococcus faecium</i>	28
<i>Staphylococcus epidermidis</i>	17
<i>Escherichia coli</i>	17
<i>Enterococcus faecalis</i>	14
<i>Staphylococcus haemolyticus</i>	7
<i>Klebsiella pneumoniae</i>	9
<i>Enterococcus durans</i>	7
<i>Lactobacillus helveticus</i>	4
<i>Enterococcus hiriae</i>	3
<i>Roseomonas genomospecies 5</i>	1
Total	107

Table 2 Primers used in this study.

Region	Sequence (5' → 3')	Product size (bp)	Reference
<i>bla_{SHV}</i>	ATTGTGCGCTTCTTTACTCGC TTTATGGCGTTACCTTTGACC	1018	13
<i>bla_{CTX-M}</i>	ATGTGCAGYACCAAGTAARGT TGGGTRAARTARGTSACCAGA	544	13
<i>bla_{TEM}</i>	ATGAGTATTCAACATTTCCTG CCAATGCTTAATCAGTGAGG	858	18

Multiplex amplification of genes *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*

Genomic DNA was extracted using the QIAamp DNA Mini QIAcube Kit (QIAGEN, Germany). The reactions were performed in a Touchgene Gradient thermal cycler FTGRAD2D (TECHNE DUXFORT, Cambridge, UK) using MasterMix PCR 1 × (Roche Diagnostics, Germany), 200 pmol of each primer and 200 ng of template DNA. Polymerase Chain Reactions of *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}* genes were conducted in multiplex amplification using the conditions recommended by Jemima and Verghese and Tofteland et al. (Table 2).^{13,18} PCR products were run in 1 × TBE buffer, pH 8.3 on horizontal electrophoresis in 1% agarose gels, visualized and photographed under UV illumination.

Sensitivity/resistance assays

Resistance/sensitivity of all strains to different antibiotics were determined using the disk diffusion method on Mueller-Hilton agar plates according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).¹⁹ The antibiotics used were: amikacin (30 µg), ampicillin (30 µg), carbenicillin (100 µg), cefalotine (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), netilmicin (30 µg), nitrofurantoin (300 µg) and, trimethoprim/sulfamethoxazole (25 µg). Antibiotics used in this test are those that are commonly prescribed prophylactic against Gram positive and Gram-negative bacteria. *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 strains were used as controls. Results were interpreted as susceptible or resistant by measuring the diameter of inhibition zone according to the criteria stipulated by the CLSI.²⁶

Results

Multiplex amplification of genes *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*

One hundred and seven bacterial strains isolated of Umbilical Cord Blood Unit for hematopoietic transplant were identified by analysis of sequence of 16S rRNA and subjected to multiplex amplification by PCR in order to detect encoding genes β -lactamases (*bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*). Twenty-eight (26.1%) strains were positive to *bla_{SHV}* gene, 40 (37.3%) to *bla_{CTX-M}* gene and 48 (54.2%) *bla_{TEM}* gene.

Table 3 Genotype detected of antibiotic resistance in bacteria strains isolated from Umbilical Cord Units for transplant.

Tested strains		Detected genotype					
		<i>bla</i> variants detected			<i>bla</i> combinations detected		
		<i>bla</i> _{SHV}	<i>bla</i> _{CTX-M}	<i>bla</i> _{TEM}	<i>bla</i> _{SHV/TEM}	<i>bla</i> _{SHV/CTX}	<i>bla</i> _{CTX/TEM}
<i>Enterococcus faecium</i>	28	5 (17.8)	9 (32.1)	10 (35.7)	4 (14.2)	0 (0)	2 (7.1)
<i>Staphylococcus epidermidis</i>	17	5 (29.4)	4 (23.5)	6 (35.2)	1 (5.8)	0 (0)	2 (11.7)
<i>Escherichia coli</i>	17	6 (35.2)	10 (58.8)	11 (64.7)	1 (5.8)	2 (11.7)	1 (5.8)
<i>Enterococcus faecalis</i>	14	2 (14.2)	5 (35.7)	6 (42.8)	2 (14.2)	0 (0)	1 (7.1)
<i>Staphylococcus haemolyticus</i>	7	2 (28.5)	4 (57.1)	3 (42.8)	1 (14.2)	0 (0)	2 (28.5)
<i>Klebsiella pneumoniae</i>	9	4 (44.4)	4 (44.4)	6 (66.6)	2 (22.2)	1 (11.1)	1 (11.1)
<i>Enterococcus durans</i>	7	2 (28.5)	2 (28.5)	3 (42.8)	0 (0)	0 (0)	0 (0)
<i>Lactobacillus helveticus</i>	4	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
<i>Enterococcus hiriae</i>	3	1 (33.3)	2 (66.6)	1 (33.3)	0 (0)	0 (0)	1 (33.3)
<i>Roseomonas gensp 5</i>	1	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
Total	107	28 (26.1)	40 (37.3)	48 (44.8)	12 (11.2)	3 (2.8)	10 (9.34)

R, resistant; S, sensible.

The numbers indicated in parentheses is the percentage (%) of *bla*-gene detection.

Twelve strains were positive (11.2%) to *bla*_{SHV/TEM}, 3 (2.8%) to *bla*_{SHV/CTX} and 10 (9.34%) to *bla*_{CTX/TEM}. A summary of genotype detected in all strains is shown in Table 3. The genes were predominantly detected in *Enterobacteriaceae* (*E. coli* and *K. pneumoniae*), *Staphylococcaceae* (*S. epidermidis* and *S. haemolyticus*) and *Enterococcaceae* (*E. faecium* and *E. faecalis*) family members (Fig. 1). The ATCC strains (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as negative controls of amplification (data not shown).

Resistance to antimicrobials test

The strains showed differences in susceptibility and resistance to the antimicrobials tested. Sulfonamides, folate antagonists, nitrofurans, and first generation of cephalosporins had the best antimicrobial activity. The broad-spectrum penicillins, third generation of cephalosporins, aminoglycosides, and fluoroquinolones showed lower activity (Fig. 2).

Statistical analysis

For statistical analysis, the following was considered: null hypothesis (Ho)=independent variable (presence of *bla* genes in different strains), alternative hypothesis (H1): dependent variable (*bla* genes related to the strain or type of *bla* gene). Considering $p > 0.05$, the null hypothesis is accepted and the alternative hypothesis is rejected. p values of 0.242 (*bla*_{SHV}), 0.242 (*bla*_{CTX-M}) and 0.254 (*bla*_{TEM}) were calculated. As in all cases the result was greater than 0.05, therefore be concluded that the results are significantly different at 95% confidence, indicating that there is no relationship between bacterial genera and the presence of the *bla* genes.

Discussion

The contamination of umbilical cord blood for transplant in the majority of cases occurs at the time of collection and processing or thawing of hematopoietic stem cells prior

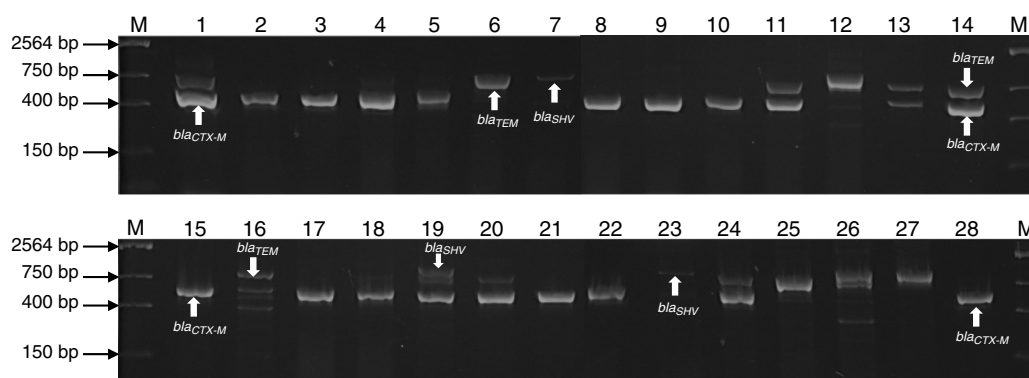


Figure 1 Agarose gel electrophoresis representative of the PCR products of encoding genes β -lactamases (*bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M}) of bacterial strains isolated of UBCU for transplant. Lanes: M: Molecular size marker (One lambda) Thermo Fisher Scientific, Brand, 1–28: PCR products of *bla*_{TEM} (858 bp), *bla*_{SHV} (1018 bp), *bla*_{CTX-M} (544 bp) in some strains.

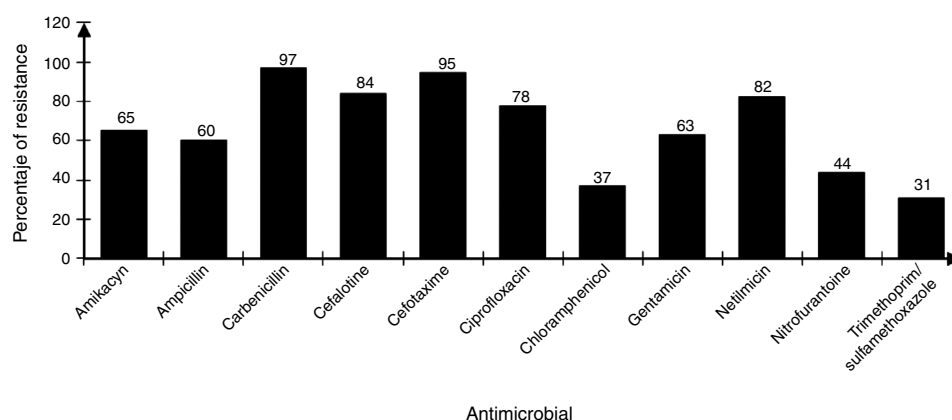


Figure 2 Percentage of resistance to antimicrobial in strains isolated from UCBU.

to transplantation.¹⁷ In general, the increased incidence of contamination in placental blood occurs in proportion with the increase in handling steps of the final UCBU, such as removing (purging) the excess red blood cells by not automated means and cryopreservation procedures, between others.^{20,26} The bacterial strains analyzed in this work are mainly family members *Enterobacteriaceae*, *Enterococcaceae* and *Staphylococcaceae*; therefore the source was: digestive tract and skin flora respectively. This allows the identification of critical points, from the time of collection of the UCB in the operating room to prevent bacterial contamination. Valuable information about the strains identified in UCBU for transplant, is related to resistance to antibiotics since from this depends the patient safety. As mentioned, before patients exposed to transplant protocols with HSC are subject to a preconditioning program (immunosuppression) to prevent graft rejection. Therefore, the patient is exposed to opportunistic infections caused by bacteria that are generally innocuous, potentially pathogenic or virulent. Knowledge about this resistance and its genotype, provides an opportunity to act efficiently in an emergency providing effective antimicrobial treatment. The identification of resistance phenotype by disk diffusion method and genotype by multiplex amplification by PCR in all strains, showed the presence of genes in some strains that are phylogenetically distant from the family *Enterobacteriaceae* (*Enterococcaceae*, *Staphylococcaceae*, *Acetobacteraceae* and *Lactobacteriaceae*). The presence of phylogenetically distant genes in bacteria has been previously reported; we speculate that the presence of these genes reported in the *Enterobacteriaceae* family is due to events of horizontal transfer of genetic material between bacteria by specific mechanisms (conjugation, transduction and transformation). These phenomena are not new in their nature and less on bacteria enteric origin, where it is known that DNA transfer is incredibly high and clearly explains the presence of DNA transfer events. Previous studies demonstrated the transfer of bacterial DNA in the human digestive tract, therefore we suppose that this phenomenon is the reason of the presence of this type of genes in the isolates. Resistance to β -lactamics antibiotics is mainly attributable to the production of β -lactamases enzymes. The β -lactamases spread spectrum enzymes (ESBL) represent today a great risk due to a broad spectrum to antibiotic inactivation usually available for the treatment

of infections. These strains producing β -lactamases exhibit hydrolytic activity against penicillin and cephalosporins. Since its initial description more than 300 different ESBL have been identified, and most of them belong to the TEM, SHV and CTX-M families (Paterson et al., 2005).²¹ The analysis of the presence of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} genes in 107 bacterial strains isolated from UCBU indicated the presence of *bla*_{TEM} gene (44.8%) followed by *bla*_{CTX-M} (37.3%) and finally *bla*_{SHV} (26.1%). These results are comparable to those obtained in tests of sensitivity/resistance and show that cefotaxime is the least effective antibiotic against these strains (95%) (Fig. 2); this is a third generation cephalosporin with an oximino group, being the most susceptible to inactivation by the β -lactamases.²² In the case of β -lactamics, ampicillin has a low level of activity, 60% of strains were resistant to this compound; *bla* gene has been reported as being responsible for ampicillin resistance in *E. coli* and other enterobacterias.¹⁷ Moreover aminoglycosides have limited activity against most ESBL producing strains being gentamicin the least active within this family (63%).²³ In the case of amikacin, another aminoglycoside, antibiotic presented greater activity and only 65% of the strains were resistant. Similar results have been reported to amikacin before.²⁴ The emergence of such resistance is mainly due to over-consumption of antibiotics, as well as the natural existence of genetic material and their acquisition by horizontal transfer among bacteria, this is the case of the presence of encoders as TEM, SHV and CTX-M β -lactamases.²⁵ The presence of genes *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} was demonstrated in all strains tested. It was determined that third-generation cephalosporins and β -lactamics such as cefotaxime and ampicillin, respectively, showed low effective activity against the strains tested. Instead, chloramphenicol and amikacin proved less susceptible to inactivation by β -lactamases. The reduction in the spread of resistance genes mediated requires the identification of the genes involved with the purpose of controlling the movement of this mechanism of resistance.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors declare that they have no conflict of interests.

References

1. Szilvassy SJ, Review article The biology of hematopoietic stem cells. *Arch Med Res.* 2003;34:446–60.
2. Porada CD, Atala AJ, Almeida-Porada G. The hematopoietic system in the context of regenerative medicine. *Methods.* 2015.
3. Gunsilius E, Gastl G, Petzer AL. Hematopoietic stem cells. *Biomed Pharmacother.* 2001;55:186–94.
4. Frey MA, Guess C, Allison J, et al. Umbilical cord stem cell transplantation. *J Semin Oncol Nurs.* 2009;25:115–9.
5. Shihabuddin LS, Aubert I. Stem cell transplantation for neurometabolic and neurodegenerative diseases. *Neuropharmacology.* 2010;58:845–54.
6. López-Martínez A, Chávez-Muñoz C, Granados J. Función biológica del complejo principal de histocompatibilidad. *Rev Invest Clin.* 2005;57:132–41.
7. Martínez-Álvarez JC. El papel del complejo principal de histocompatibilidad en el trasplante de células progenitoras hematopoyéticas. *Rev Med Inst Mex Seguro Soc.* 2005;43:87–9.
8. Jaime Fagundo JC, Dorticós Balea E, Pavón Morán V, et al. Aspectos inmunológicos del trasplante de células progenitoras hematopoyéticas. *Rev Cuba Hematol Inmunol Med Transf.* 2006;22.
9. Rifón JJ. Trasplante de progenitores hemopoyéticos. *An Sist Sanit Navar.* 2006;29:137–52.
10. Klein MA, Kadidlo D, McCullough J, et al. Microbial contamination of hematopoietic stem cell products: incidence and clinical sequelae. *Biol Blood Marrow Transpl.* 2006;12:1142–9.
11. Kozłowska-Skrzypczak M, Bembnista E, Kubiak A, et al. Microbial contamination of peripheral blood and bone marrow hematopoietic cell products and environmental contamination in a stem cell bank: a single-center report. *Transpl Proc.* 2014;46:2873–6.
12. Bello-López JM, Fernández-Rendón E, Curiel-Quesada E. *In vivo* transfer of plasmid pRAS1 between *Aeromonas salmonicida* and *Aeromonas hydrophila* in artificially infected *Cyprinus carpio* L. *J Fish Dis.* 2010;33:251–9.
13. Jemima SA, Verghese S. Multiplex PCR for bla(CTX-M) & bla(SHV) in the extended spectrum beta lactamase (ESBL) producing gram-negative isolates. *Indian J Med Res.* 2008;128:313–7.
14. Dullius Almeida I, Schmalfussb T, Marise Röhsigb L, et al. Autologous transplant: microbial contamination of hematopoietic stem cell products. *Braz J Infect Dis.* 2012;16:345–50.
15. Moraga MR, Santander PE, Arias CT, et al. Integrines y su relación con el fenotipo de resistencia en bacilos gramnegativos aislados en el Hospital Torres Galdames de Iquique, Chile. *Rev Chil Infectol.* 2007;24:384–90.
16. Pitout JDD, Nordmann P, Laupland KB, et al. Emergence of Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs) in the community. *J Antimicrob Chemother.* 2005;56:52–9.
17. Bello-López JM, Noguerón-Silva J, Castañeda-Sánchez I, et al. Molecular characterization of microbial contaminants isolated from Umbilical Cord Blood Units for transplant. *Braz J Infect Dis.* 2015, <http://dx.doi.org/10.1016/j.bjid.2015.07.005>.
18. Tofteland S, Haldorsen B, Dahl KH, et al. Effects of phenotype and genotype on methods for detection of extended-spectrum β -lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. *J Clin Microbiol.* 2007;45:199–205.
19. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). Approved standard: M2. Performance standards for antimicrobial disk susceptibility tests. Wayne, PA, USA: CLSI; 2002.
20. García CP. Recomendaciones para el estudio microbiológico de tejidos preservados para implantes. *Rev Chil Infectol.* 2004;21:102–16.
21. Paterson DL, Bonomo RA. Extended-spectrum β -lactamases: a clinical update. *Clin Microbiol Rev.* 2005;18:657–86.
22. Ferrández Quirante O, Grau Cerrato S, Luque Pardos S, et al. Tratamiento de las infecciones por microorganismos productores de betalactamasas de espectro extendido: un nuevo reto terapéutico. *Farm Hosp.* 2005;29:351–3.
23. Stürenburg E, Mack D. Extended-spectrum β -lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. *J Infect.* 2003;47:273–95.
24. Spanu T, Luzzaro F, Perilli M, et al. Occurrence of extended-spectrum β -lactamases in members of the family *Enterobacteriaceae* in Italy: Implications for resistance to β -lactams and other antimicrobial drugs. *Antimicrob Agents Chemother.* 2002;46:196–202.
25. Philippon A, Arlet G, Jacoby GA. Plasmid-Determined AmpC-Type β -lactamases. *Antimicrob Agents Chemother.* 2002;46:1–11.
26. Acosta-Pérez G, Ibáñez-Cervantes G, Bello-López JM, et al. Structural diversity of class 1 integrons in multiresistant strains of *Escherichia coli* isolated from patients in a hospital in Mexico city. *Curr Microbiol.* 2015;71:501–8.