Heliyon

Received:
22 March 2016
Revised:
3 June 2016
Accepted:
8 June 2016

Heliyon 2 (2016) e00121

In vivo efficacy of humanized high dose meropenem and comparators against *Pseudomonas aeruginosa* isolates producing verona integron-encoded metallo-β-lactamase (VIM)

Islam M. Ghazi[a], **Jared L. Crandon**[a], **Emil P. Lesho**[b], **Patrick McGann**[b], **David P. Nicolau**[a,c,*]

[a] *Center for Anti-infective Research and Development, Hartford Hospital, Hartford, CT, USA*

[b] *Walter Reed Army Institute of Research, Silver Spring, MD, USA*

[c] *Division of Infectious Diseases, Hartford Hospital, Hartford, CT, USA*

* Corresponding author at: Center for Anti-infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford, CT 06102, USA.

E-mail address: david.nicolau@hhchealth.org (D.P. Nicolau).

Abstract

Introduction: We aimed to describe the *in vivo* efficacy of meropenem, in addition to cefepime and levofloxacin as comparators against VIM-producing *Pseudomonas aeruginosa* and compare the findings to our previous observations with Enterobacteriaceae.

Methods: Eight clinical *P. aeruginosa* isolates with meropenem MICs from 4 to 512 mg/L were studied in a murine neutropenic thigh infection model. Animals were treated with doses of the antibiotics to simulate the human exposure of meropenem 2 g q8 h 30-min infusion, cefepime 2 g q8 h 30-min infusion and levofloxacin 500 mg q24 h. After 24 hours, the animals were euthanized and efficacy was

http://dx.doi.org/10.1016/j.heliyon.2016.e00121

2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

calculated as the change in thigh bacterial density ($\log_{10}$ CFU) relative to the starting inoculum (0 h).

Results: As expected, levofloxacin was ineffective against all isolates due to their resistant phenotype (8 to>64 mg/L). Cefepime also showed minimal activity against all isolates consistent with its failure to achieve pharmacodynamic target exposures due to high MICs of 32 to>512 mg/L. In the presence of low MICs (4 to 16 mg/L), the *f*T> MIC of meropenem was sufficiently high to result in CFU reductions. However, conflicting activity was noted for isolates with MICs = 128 mg/L that possessed the same enzymatic profile, suggesting that other mechanisms of resistance are responsible for driving CFU outcomes. No activity was noted for organisms with a meropenem MIC = 512 mg/L.

Conclusion: Unlike previous observations with MBL-producing Enterobacteriaceae that showed discordance between *in vitro* resistance and *in vivo* efficacy in the murine infection model, we found that the efficacy of humanized cefepime and meropenem was generally concordant with the phenotypic profile of VIM-producing *P. aeruginosa*.

Keyword: Microbiology

1. Introduction

Infections caused by multi-drug resistant (MDR) pathogens, including *Pseudomonas aeruginosa* are increasing globally. *P. aeruginosa* is often thought of as the prototypic MDR organism as it is capable of developing resistance by utilizing both non-enzymatic, such as modification of the outer membrane protein OprD, and enzymatic processes, which include mutations in chromosomal genes or transferable resistance determinants such as metallo-β-lactamases (MBLs) and extended spectrum β-lactamases (ESBL) [1]. The spread of MBLs among *P. aeruginosa* and *Enterobacteriaceae* has been rising in the recent years. Among the clinically relevant MBL enzymes is VIM, which was first recovered in Italy in 1997 and manifested resistance to several β-lactam antibiotics, including imipenem with a minimum inhibitory concentration (MIC) of>128 mg/L [2]. As a result of these emerging resistance trends and the limited therapeutics options against these MBLs, investigations assessing the clinical implications of resistance as defined by the current *in vitro* criteria are required. To this end, previously conducted studies suggest that for *Enterobacteriaceae*, humanized exposures of carbapenems retain *in vivo* activity against two MBL-producers (NDM and VIM), despite resistant as defined by *in vitro* MICs [3, 4]. In this study, we sought to compare and contrast the effect of VIM production in Enterobacteriaceae and *P. aeruginosa* by characterizing the efficacy of meropenem and comparators, levofloxacin and cefepime, against VIM-producing *P. aeruginosa*.

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2. Methods

The ethical approval was obtained from Institutional Animal Care and Use Committee before the start of the study. Commercially available levofloxacin (Akorn Inc, Lake Forest, IL), meropenem (Hospira Inc, Lake Forest, IL) and cefepime (Sagent, Schaumberg, IL) were acquired from the Hartford Hospital Pharmacy Department (Hartford, CT) to be used during *in vivo* experimentation. All antibiotics were reconstituted according the manufacturers' recommendations and further diluted to the required concentration using normal saline shortly before the experiment.

A total of eight clinical *P. aeruginosa* isolates were tested (Table 1). All isolates were frozen at −80 °C in double strength skim milk (Remel, Lenexa, KS) and cultured twice consecutively on trypticase soy agar with 5% sheep blood (BD Biosciences, Sparks, MD) and incubated at 35 °C for 18–24 hours prior to experimentation. Levofloxacin, meropenem, and cefepime MICs were determined using broth microdilution in accordance with Clinical Laboratory Standards Institute (CLSI) methodology using standard analytical powder (Sigma-Aldrich, St. Louis, MO) [5].

Table 1. List of the tested isolates with their respective genotypes and MICs.

Clinical isolates	β-lactamases encoded	MIC (mg/L)		
		LEV	FEP	MER
PSA 1550	VIM-1, OXA-10, OXA-50	32	64	4
PSA 1480	VIM-2, OXA-2, OXA-10, OXA-50,	64	32	16
PSA 1475	VIM-1, OXA-50,	8	>512	64
PSA 1465	VIM-1, OXA-50,	>64	512	128
PSA 1478	VIM-1, OXA-50,	>64	256	128
PSA 1466	VIM-2 OXA-4, OXA-50,	>64	256	512
PSA 1476	VIM-1, OXA-50,	16	>512	512
PSA 1549	VIM-6, OXA-10, OXA-50	>64	256	512

PSA: P. aeruginosa.
LEV; levofloxacin, FEP; cefepime, MER; meropenem.
European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints:
Levofloxacin; susceptible ≤ 1 mg/L.
Cefepime; susceptible ≤ 8 mg/L.
Meropenem; susceptible ≤ 8 mg/L.
Clinical Laboratory Standards Institute (CLSI) breakpoints:
Levofloxacin; susceptible ≤ 2 mg/L.
Cefepime; susceptible ≤ 8 mg/L.
Meropenem; susceptible ≤ 4 mg/L.

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license
(http://creativecommons.org/licenses/by-nc-nd/4.0/).

Whole genome sequencing was performed using an Illumina MiSeq desktop sequencer at Walter Reed Army Institute of Research (Silver Spring, MD, USA), as previously described [6, 7]. Multilocus sequence type profiles were determined based on database maintained by the Institut Pasteur (Paris, France) and by NCBI (Bethesda, MD, USA), respectively. The study was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital, Hartford, CT and animals were kept in consistence with National Research Council's recommendations and given nutrition *ad libitum.* ICR mice with an average weight of 22 g were obtained from Harlan Laboratories (Indianapolis, IN) and utilized throughout. Neutropenia was induced with cyclophosphamide 150 mg/kg and 100 mg/kg at 4 days and 1 day prior to inoculation, respectively. In addition, uranyl nitrate 5 mg/kg was administered three days prior to inoculation to produce a controlled degree of renal impairment. The study animals were inoculated in each thigh with 0.1 ml of bacterial suspension containing 10^7 CFU/ml of the test isolate in normal saline and antibiotic administration started 2 hours later. Humanized doses of levofloxacin 500 mg q24 h designed to achieve an AUC of 42–53 mg.h/L [8], meropenem 2 g q8 h as 30-min infusion [9] and cefepime 2 g q8 h as 30-min infusion [10], as previously developed and validated by our group, were administered as 0.2 ml subcutaneous injections to groups of three animals over 24 hours. The β-lactams regimens were designed to simulate the pharmacodynamic profile observed in humans given these regimens (Table 2). As required to optimize the use of animals in research, repeated PK studies were not done in our current investigation because exposure profile validation was defined previously in our laboratory on the same mice species. A group of three mice was harvested at the beginning of dosing to serve as 0 hour controls and another group was given 0.2 ml subcutaneous normal saline as frequently as the most dosed antibiotic for each isolate. At the end of 24-hour study period, treatment and control animals

Table 2. Expected free drug time above the minimum inhibitory concentration (MIC) at different values of MIC for cefepime and meropenem.

	*f*T> MIC(%)	
MIC (μg/ml)	**Cefepime**	**Meropenem**
4	100	58
8	83	45
16	65	30
32	38	18
64	20	5
128	1	0

Adapted from [9] and [10].

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

were euthanized by CO_2 exposure followed by cervical dislocation; thighs were excised from sacrificed animals, homogenized in 5 ml of normal saline (mini bead-beater, BioSpec Products, Bartlesville, OK), and serially diluted and plated on trypticase soy agar with 5% sheep blood for counting of bacterial densities. Efficacy was calculated as the change in bacterial density ($\log_{10}$ CFU) of intervention groups compared with the 0 hour control group. To assure enzyme production across experimentation, thigh homogenates from 0-hour and 24-hour control animals, as well as, meropenem treated mice were plated on agar containing meropenem concentrations equivalent to the MIC of the test strain.

3. Results

A list of study isolates along with their respective MIC and genotypes is shown in Table 1. The growth (mean ± SD) of the 0h-control group was 5.00 ± 0.36 $\log_{10}$ CFU while that of 24h-control group was 8.52 ± 0.47. Levofloxacin (Fig. 1) exhibited a lack of activity against all tested isolates as predicted based on the

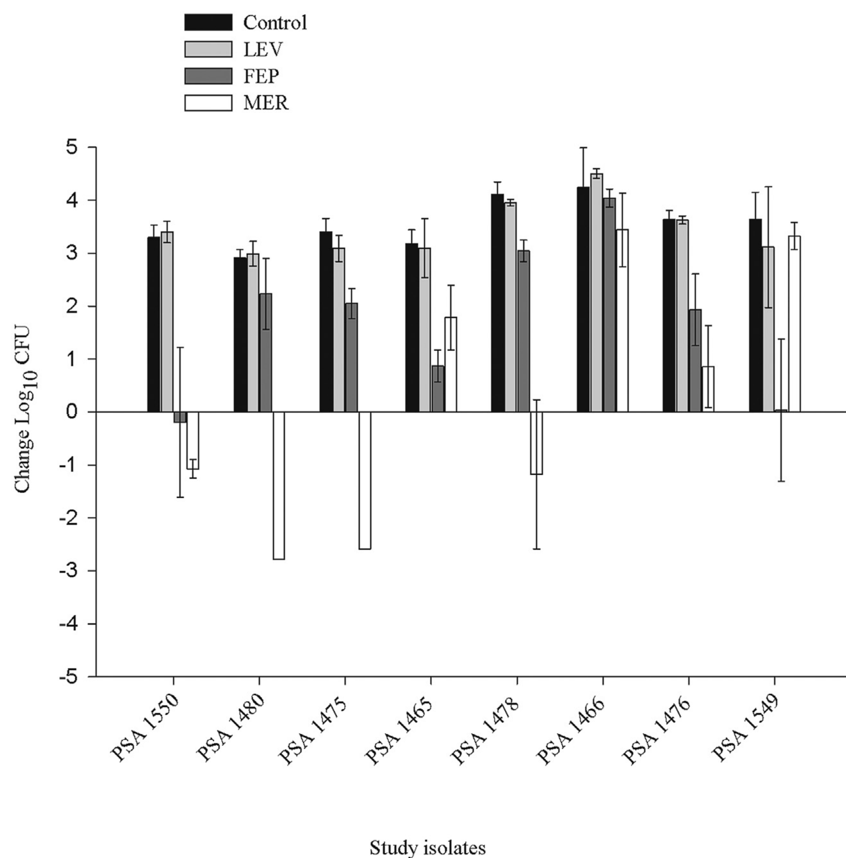

Fig. 1. Efficacy of humanized levofloxacin 500 mg q24 h, cefepime 2 g q8 h 30-min. infusion and meropenem 2 g q8 h 30-min. infusion against *P. aeruginosa* isolates producing VIM in neutropenic murine thigh model.

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

phenotypic profile of the test strains. Similarly, humanized cefepime had minimal activity against the 8 isolates, consistent with the inability of this regimen to achieve pharmacodynamic targets (Table 2). Conversely, meropenem was predictably active against PSA1550 and PSA1480 as their MIC profiles resulted in sufficiently high free drug exposures. Moreover, while the *f*T> MIC of PSA1475 was reduced (5–18%), considerable efficacy was noted for this isolate. Activity was also observed for PSA1478 (MIC = 128 mg/L, *f*T> MIC = 0–5%), on the other hand there was no activity against PSA 1465 with the same MIC. No activity was observed for the remaining 3 strains with MIC of 512 mg/L. All isolates recovered from the mice after treatment showed growth on meropenem containing plates, thus confirming the presence of enzyme mediated activity throughout the *in vivo* exposure period.

4. Discussion

The acquisition of resistance by *P. aeruginosa* with reports of emerging metallo-β-lactamases worldwide is a growing challenge in nosocomial infections and efforts are needed to describe appropriate treatment options [11]. In the current *in vivo* murine study employing humanized exposures, we found that the *in vitro* MIC of VIM-producing *P. aeruginosa* was reflective of the *in vivo* activity of levofloxacin, cefepime, and meropenem. These observations in *P. aeruginosa* were unlike what we have previously demonstrated with Enterobacteriaceae [3, 4]. As such, these current data suggest that for VIM-producing *P. aeruginosa* the expected clinical efficacy of monotherapy will be governed by the achievement of an optimized pharmacodynamic profile according to the principle driver (i.e., *f*T> MIC, AUC/MIC) for the class of compounds under consideration.

While the concept of pharmacodynamic predictability among antimicrobials is certainly not novel [12], previous studies by our group with carbapenemase-producing Enterobacteriaceae have yielded varied results, particularly for the β-lactams [3, 4, 13, 14]. Namely, while the activity of carbapenems and cephalosporins were pharmacodynamically concordant against KPC-producing strains, activity against MBL-producers (i.e., NDM or VIM), particularly for the carbapenems, was observed *in vivo*, despite elevated *in vitro* MICs. While evidence seems to suggest that MBLs have a diminished impact *in vivo*, the discordance between observations against MBL-producing Enterobacteriaceae and *P. aeruginosa* is hypothesized to be due to the myriad of other resistance mechanisms present in *P. aeruginosa*, such as porin or efflux proteins [1]. This is reflected in the current study by the observation that two isolates with the same MIC and enzymatic resistance genotypes showed opposite killing patterns *in vivo,* thus the overall contribution of the enzyme mediated resistance *in vivo* appears blunted in the presence of alternative mechanisms.

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

While we found the tested agents to be generally ineffective against resistant strains, a previous study of a single VIM-producing *P. aeruginosa* in the rat pneumonia model found that high doses of imipenem, cefepime, ceftazidime, piperacillin/tazobactam and aztreonam (MICs were 128, 64, 256, 16 and 0.25 mg/L, respectively) were effective in reducing bacterial loads *in vivo* despite *in vitro* resistance [15]. While the use of human simulated exposures is mentioned by the authors, the free-drug profile of their treatment regimens in blood and lung are not provided; therefore direct comparisons between the two studies are difficult.

The available clinical data have repeatedly shown poor outcomes for patients infected with multi-drug resistant *P. aeruginosa* producing VIM enzymes. Assessments of appropriate empiric and definitive treatment are challenging given that many of these organisms are resistant to almost all the antibiotics, except colistin [16]. Moreover, in some cases these organisms are pan-drug resistant, with combinations of colistin and β-lactams being reported as viable [17].

Unlike previous observations with MBL-producing Enterobacteriaceae, we found that the MIC of the infecting VIM-producing *P. aeruginosa* predicted *in vivo* efficacy of humanized cefepime and meropenem.

5. Conclusion

Given the high rates of resistance among these VIM-producing *P. aeruginosa* and the resultant poor pharmacodynamics profiles treatment with monotherapy does not appear to be a viable option for a large proportion of these strains and further emphasizes the need for novel compounds targeted at these difficult pathogens.

Declarations

Author contribution statement

Islam M. Ghazi, David P. Nicolau: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jared L. Crandon: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Emil P. Lesho, Patrick McGann: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license
(http://creativecommons.org/licenses/by-nc-nd/4.0/).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We acknowledge Kimelyn Greenwood, Jennifer Hull, Lucinda Lamb, Sara Robinson, Debora Santini, Pamela Tessier and Abrar Thabit (Center for Anti-Infective Research and Development, Hartford Hospital) for their assistance with animal experiments and MIC determination. VIM-producing strains were kindly provided by Multidrug-Resistant organism Repository and Surveillance Network of WRAIR and International Health Management Associates.

References

[1] D.M. Livermore, Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare, Clin. Infect. Dis. 34 (2002) 634–640.

[2] L. Lauretti, M.L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, et al., Cloning and characterization of blaVIM, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate, Antimicrob. Agents Chemother. 43 (1999) 1584–1590.

[3] D.E. Wiskirchen, P. Nordmann, J.L. Crandon, D.P. Nicolau, In vivo efficacy of human simulated regimens of carbapenems and comparator agents against NDM-1-producing Enterobacteriaceae, Antimicrob. Agents Chemother. 58 (2014) 1671–1677.

[4] I.M. Ghazi, J.L. Crandon, E.P. Lesho, P. Mc Gann, D.P. Nicolau, Efficacy of Humanized High Dose Meropenem, Cefepime and Levofloxacin against Enterobacteriaceae Isolates Producing Verona Integron-Encoded Metallo-β-Lactamase (VIM) in a Murine Thigh Infection Model, Antimicrob. Agents Chemother. 59 (2015) 7145–7147.

[5] Clinical Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing: twenty-fourth informational supplement [document M100-S25], National Committee for Clinical Laboratory Standards, Wayne, PA, 2015.

[6] P. McGann, P. Courvalin, E. Snesrud, R.J. Clifford, E.J. Yoon, F. Onmus-Leone, A.C. Ong, Y.I. Kwak, C. Grillot-Courvalin, E. Lesho, P.E. Waterman,

http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Amplification of aminoglycoside resistance gene aphA1 in Acinetobacter baumannii results in tobramycin therapy failure, MBio 5 (2014) e00915.

[7] P. McGann, E. Snesrud, A.C. Ong, L. Appalla, M. Koren, Y.I. Kwak, P.E. Waterman, E. Lesho, War wound treatment complications due to transfer of an IncN plasmid harboring blaOXA-181 from Morganella morganii to CTX-M-27-producing ST131 Escherichia coli, Antimicrob. Agents Chemother. 59 (2015) 3556–3562.

[8] C.O. Onyeji, K.Q. Bui, R.C. Owens, D.P. Nicolau, R. Quintiliani, C.H. Nightingale, Comparative efficacies of levofloxacin and ciprofloxacin against Streptococcus pneumoniae in a mouse model of experimental septicemia, Int. J. Antimicrob. Agents 12 (1999) 107–114.

[9] C.A. DeRyke, M.A. Banevicius, H.W. Fan, D.P. Nicolau, Bactericidal activities of meropenem and ertapenem against extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a neutropenic mouse thigh model, Antimicrob. Agents Chemother. 4 (2007) 1481–1486.

[10] J.L. Crandon, D.P. Nicolau, In Vivo Activity of Humanized Cefepime and Cefepime/AAI101 against Multidrug-Resistant Gram-negative Enterobacteriaceae, Antimicrob. Agents Chemother. 59 (2015) 2688–2694.

[11] P. Nordmann, L. Poirel, Emerging carbapenemases in Gram-negative aerobes, Clin. Microbiol. Infect. 8 (2002) 321–331.

[12] W.A. Craig, Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men, Clin. Infect. Dis. 26 (1998) 1–10.

[13] D.E. Wiskirchen, P. Nordmann, J.L. Crandon, D.P. Nicolau, Efficacy of humanized carbapenem and ceftazidime regimens against Enterobacteriaceae producing OXA-48 carbapenemase in a murine infection model, Antimicrob. Agents Chemother. 3 (2014) 1678–1683.

[14] M. Hagihara, J.L. Crandon, C. Urban, D.P. Nicolau, Efficacy of doripenem and ertapenem against KPC-2-producing and non-KPC-producing *Klebsiella pneumoniae* with similar MICs, J. Antimicrob. Chemother. 7 (2013) 1616–1618.

[15] S. Bellais, O. Mimoz, S. Léotard, A. Jacolot, O. Petitjean, P. Nordmann, Efficacy of beta-lactams for treating experimentally induced pneumonia due to a carbapenem-hydrolyzing metallo-beta-lactamase-producing strain of *Pseudomonas aeruginosa*, Antimicrob. Agents Chemother. 46 (2002) 2032–2034.

 http://dx.doi.org/10.1016/j.heliyon.2016.e00121
2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

[16] S. Corvec, L. Poirel, E. Espaze, C. Giraudeau, H. Drugeon, P. Nordmann, Long-term evolution of a nosocomial outbreak of *Pseudomonas aeruginosa* producing VIM-2 metallo-enzyme, J. Hosp. Infect. 68 (2008) 73–82.

[17] M.E. Falagas, I.A. Bliziotis, S.K. Kasiakou, G. Samonis, P. Athanassopoulou, A. Michalopoulos, Outcome of infections due to pandrug-resistant (PDR) Gram-negative bacteria, BMC Infect. Dis. 5 (2005) 24.

 http://dx.doi.org/10.1016/j.heliyon.2016.e00121

2405-8440/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).