ANSWERING REVIEWERS

June 28, 2016

Dear Editor,

Please find enclosed the edited manuscript and an electronic copy of the full-text manuscript in Word format (file name -Revised manuscript.docx)

Title: Global epidemiology of Hepatitis C Virus (HCV) infection: an up-date of the distribution and circulation of HCV genotypes

Author: Arnolfo Petruzziello, Samantha Marigliano, Giovanna Loquercio, Anna Cozzolino, Carmela Cacciapuoti

Manuscript No: 26551

The manuscript has been improved according to the suggestions of reviewers:

Format has been updated according to the editor's suggestions:

1. **Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).**

The article has been translated and corrected by a professional native English translator.

2. Name of Journal: ***World Journal of Gastroenterology***
ESPS Manuscript NO:.....
Manuscript Type:.....

All the above information have been enclosed

3. **Please revise and perfect your manuscript according to peer-reviewers' comments**
Title has been changed in :" Global epidemiology of Hepatitis C Virus (HCV) infection: an up-date of the distribution and circulation of HCV genotypes"

4. **Audio core tip:**

In order to attract readers to read your full-text article, we request that the author make an audio file describing your final core tip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information.
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6. **Please provide all authors abbreviation names and manuscript title here.** ***World J Gastroenterol*** **2016; In press**

Done.

7. Abbreviations and acronyms are often defined the first time they are used within the main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention.

Done.

8. Please check that there are no repeated references! Please add PubMed citation numbers and DOI citation to the reference list and **list all authors.**Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) DOI (http://www.crossref.org/SimpleTextQuery/) (Please begin with DOI: 10.**)

The manuscript has been improved according to the suggestions of reviewers:

Reviewer's code: 02528622

Comments To Authors:
Very interesting paper. The analysis performed by the authors maximized the extraction of meaningful information from available literature. While subgenotyping information could not be assessed, the current HCV genotype distribution is relevant for the implementation of adequate control measures aimed to prevent virus spread.

Reviewer's code: 00225318

Comments To Authors:
The introduction provides a good, generalized background of the topic that quickly gives the reader an appreciation of the wide search of updated information regarding the main subject. However, to make the introduction more substantial, the author may wish to provide references and technical explanation of the current commercial methods available to genotype. I think the motivations for this study it's clear enough, but it will improve if the author may wish to add the usefulness of the correct determination of the HCV genotype and subtype regarding the new DAA, providing examples of mutation associated with resistance. The searching methodology is appropriate for the study, although the author should probably provide more information about how the genotype and viral load was determined in each region. Maybe the author would beneficiate of an increase in data to analyze if he uses the result regarding of what he calls "non-representative population", and compare it with the rest of the population. Conclusions are sound, and give the reader a correct appreciation of the main subject in the article,

Due to the potential clinical implications of the different HCV genotypes, reliable methods are required to classify different strains of viral genome from primary patient isolates. Following the right suggestion of the reviewer In the Introduction Section we have added a large paragraph concerning the different methods available for HCV genotyping at pages 6-7 from line 130 to 156 with 5 new references:

"The most accurate method for determining genotype involves the direct sequencing of a specific polymerase chain reaction (PCR)-amplified portion of the viral genome (the NS5, core, E1 and 5′ UTR regions) obtained from a patient sample, followed by phylogenetic analysis [18-20]. Sequencing, often of multiple regions of the viral genome, in combination with the generation of phylogenetic trees, can give an accurate classification of primary patient isolates [17,21]. There are several methods used to determine HCV genotypes: restriction fragment length polymorphisms (RFLP), which utilizes restriction enzymes to recognize genotype-specific cleavage sites in a PCR-amplified DNA fragment [22] and whose sensitivity and specificity seems to need further investigations [23], Line probe assays (LiPA) [14–16], and, more recently, kinetic amplification with dissociation analysis [24-25], whose reproducibility will need surely more investigations in the future.

The most common method is surely Lipa in which PCR amplicons hybridize to genotype-specific probes immobilized on nitrocellulose strips or a 96-well microplate and are visualized using colorimetric chemistry. Simple interpretation of the banding pattern determines the viral genotype or the presence of known mutations. Genotype-specific antibodies able to recognize the NS4 region of HCV have also been exploited [20], even if this type of serological genotyping still lacks specificity and sensitivity with huge limits of its clinical applications. Anyway, although all of these

methods are able to identify the major groups of HCV, only direct nucleotide sequencing is actually efficient in discriminating among subtypes, detecting known mutations and elucidating new polymorphisms that may have clinical implications [20].”

The methodology used for the evaluation of prevalence, viraemic rate and genotype distribution in each region has been clarify at page 9 from line 204 to 211:

“The average HCV prevalence for each continent was calculated by dividing the sum of data reported from each region to the total number of countries within the region. The first- and second generation immunoassay tests which usually provide false-positive results overestimating the total infected population [37,38] were not used to estimate the country's HCV prevalence, selecting only studies in which it was used a third generation test. Similarly, the genotyping and the viraemic rate was obtained only by considering studies in which LiPa test and a well described PCR RT system (range of sensitivity and linearity) was used.”

Data from non representative population were also included as required by the reviewer (page 9- line from 215 to 220):

The infected general population was composed of high-risk groups [e.g. people who inject drugs (PWID’s), dialysis patients, haemophiliacs, minority ethnic groups, etc] as well as non-high-risk groups that contracted the disease through contact with infected blood (e.g. nosocomial infections, dental procedures, etc.). Studies with a sample size of less than 1000 and studies published prior to 2000 or not in English were excluded from the analysis.”

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology.*

Sincerely yours,

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