



## REVIEW

# REVISED Investigating colistin drug resistance: The role of high-throughput sequencing and bioinformatics

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## Abstract

Bacterial infections involving antibiotic-resistant gram-negative bacteria continue to increase and represent a major global public health concern. Resistance to antibiotics in these bacteria is mediated by chromosomal and/or acquired resistance mechanisms, these give rise to multi-drug resistant (MDR), extensive-drug resistant (XDR) or pan-drug resistant (PDR) bacterial strains. Most recently, plasmid-mediated resistance to colistin, an antibiotic that had been set apart as the last resort antibiotic in the treatment of infections involving MDR, XDR and PDR gram-negative bacteria has been reported. Plasmid-mediated colistin resistant gram-negative bacteria have been described to be PDR, implying a state devoid of alternative antibiotic therapeutic options. This review concisely describes the evolution of antibiotic resistance to plasmid-mediated colistin resistance and discusses the potential role of high-throughput sequencing technologies, genomics, and bioinformatics towards improving antibiotic resistance surveillance, the search for novel drug targets and precision antibiotic therapy focused at combating colistin resistance, and antibiotic resistance as a whole.

## Keywords

Antibiotic resistance, Colistin resistance, Pan-drug resistance, Gram negative bacteria, Genomics, Bioinformatics



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polymyxins, especially in *Acinetobacter* spp.<sup>14,15</sup>. However, a two-step mechanism has been described to elucidate their possible mechanism of action<sup>13,14</sup>.

The two-step mechanism involves i) initial binding to and permeabilization of the outer membrane and ii) the destabilization of the cytoplasmic membrane of the bacteria<sup>12–14</sup>. As a consequence, colistin functions by intercalating into the inner membrane following diffusion from the outer membrane across the periplasm and consequently causing the formation of pores, a phenomenon that results in bacterial lysis, which follows initial binding to bacterial surfaces<sup>12–14</sup>. Initial binding of colistin to the bacterial surface chiefly depends on the electrostatic interaction between the positively-charged colistin and the negatively charged phosphate group of lipid A, an endotoxic component on the lipopolysaccharide localised on the outer leaflet of the bacterial outer membrane<sup>12,14</sup>.

The modifications of the lipid A which reduce and/or abolish the initial charge-based interaction of the polymyxins with bacteria and also the addition of either/or the 4-amino-4-deoxy-L-arabinose (L-Ara4N) and the phosphoethanolamine (PEtn) that ultimately form the basis of colistin resistance in bacteria are mediated by chromosomally encoded genes<sup>13–16</sup>. These genes are involved in the modulation of two-component regulatory systems; PmrA/PmrB and PhoP/PhoQ and *mgrB*, a negative regulator of the PhoP/PhoQ signaling system<sup>13–16</sup>.

Although initially thought that this resistance could not be spread from cell to cell (plasmid-mediated)<sup>16</sup>, currently studies have shown otherwise. These have alluded transfer of colistin resistance among bacteria via plasmids in horizontal gene transfer<sup>4,16,17</sup>.

### Pan-drug resistance and characteristics of colistin-resistant gram-negative bacteria

The treatment of infections involving antibiotic-resistant gram-negative bacteria has become increasingly difficult overtime, a factor that has greatly contributed to high morbidity, mortality and high costs of health care<sup>18,19</sup>.

Currently, antibiotic resistance in these bacteria spans across several classes but likely follows a precise hierarchy of acquisition; this is mostly characterized by acquisition of “enhanced resistance” against more potent antibiotics following primary acquisition of “weaker resistance” against the less potent antibiotics alongside intrinsic resistance mechanisms in these bacteria, a trend that follows a Darwin’s like fashion<sup>20–23</sup>. These changes are a function of horizontal gene transfer, via conjugation, transformation and transduction<sup>24–27</sup>.

Resistance in gram-negative bacteria has been seen to transit from being mediated by the extended spectrum  $\beta$ -lactamases, a group of enzymes that can be disseminated among bacteria<sup>28,29</sup>; these chiefly confer resistance against broad-spectrum cephalosporins. However, they also confer resistance to penicillins, monobactams and some carbapenems, particularly the *Klebsiella pneumoniae* carbapenemase, KPC<sup>28,30,31</sup>. In the same hierarchy are AmpC  $\beta$ -lactamases that form another group of  $\beta$ -lactamases, derived from older broad spectrum  $\beta$ -lactamases. These provide

an even more extended activity that includes resistance against the cephamycins alongside resistance to penicillins, monobactams and cephalosporins<sup>32–34</sup>. These enzymes have in recent times been shown to not only be limited to being encoded on the chromosomes of bacteria, but have also been documented to have the potential of being disseminated via plasmids in horizontal gene transfer<sup>28,34,35</sup> and also to co-exist with the extended spectrum  $\beta$ -lactamases<sup>36,37</sup>; factors that have made these bacteria “better resistant” to antibiotics. Next in the hierarchy are the carbapenemases, these enzymes are chiefly acquired in horizontal gene transfer and confer resistance to carbapenems alongside resistance to penicillins, broad-spectrum cephalosporins including cefepime, a fourth-generation cephalosporin, monobactams, aminoglycosides, quinolones and fluoroquinolones<sup>28,38</sup>. The development of resistance mediated by these enzymes to the different classes of antibiotics in these bacteria has been attributed to various factors among which is their use in therapy. This has not only abetted maintenance of resistance via selecting for resistance to these antibiotics in these bacteria but has also created a gap, a need for alternative antibiotics in therapy to replace the penicillins,  $\beta$ -lactams, carbapenems and the other classes of antibiotics used in the treatment of infections involving the drug-resistant gram-negative bacteria<sup>4,16</sup>.

Colistin, a polypeptide antibiotic, a relatively old antibiotic, has been currently relied upon to provide the ultimate line of refuge against infections caused by antibiotic-resistant gram-negative bacteria despite its previously documented impacts on health<sup>4,16</sup>. Colistin also appears to offer a choice in the face of almost no new antibiotics in production pipelines<sup>4,16,17</sup>.

Worryingly, the use of colistin is under threat due to the emergence of plasmid-mediated colistin resistance involving *mcr* gene families<sup>4,16</sup>. This provides a new challenge as bacteria that express these resistance genes assume the lead in the antibiotic resistance hierarchy and are distinctively XDR or worse PDR<sup>16,39–42</sup>.

Molecular studies previously done have reported colistin resistant gram-negative bacteria to also be resistant to an array of antibiotics<sup>43,44</sup>. These bacteria have also been reported to carry plasmids that have been found to carry alongside colistin resistance genes,  $\beta$ -lactamases<sup>43,44</sup>, carbapenemase encoding genes<sup>45</sup> and genes that code for resistances to other antibiotic classes that may include quinolones, fluoroquinolones and aminoglycosides<sup>13</sup>. Additionally, the carriage of *mcr*-1 has been documented as a possible indicator of resistance to the third generation cephalosporins and carbapenems<sup>38,44</sup>. Furthermore, these genes have been found to be co-carried with other resistance determinants in plasmids<sup>13,44,46,47</sup>; these genes represent a novel mechanism of antibiotic resistance in bacteria and a threat to the existing antibiotic therapy. Worsening the situation is the ability of selection for colistin resistance via the use of the extended-spectrum cephalosporins. Additionally, the use of tetracycline and sulphonamides has also been reported to contribute to the dissemination of colistin mobile gene carrying plasmids<sup>44,46</sup>. Also, worth noting is plasmids that carry colistin resistance genes have also been found to mostly carry other antibiotic resistant genes<sup>13,44–46</sup>.

## The role of high-throughput sequencing technologies and bioinformatics

Advances in technology including the rapidly growing field of genomics, are transforming clinical medicine<sup>48</sup> and high-throughput sequencing technology (HTS) is increasingly being used in clinical microbiology<sup>49</sup>. HTS, with relatively simple benchtop technology and efficient genomic library preparation protocols, has significantly improved the capacity to perform low-cost, efficient whole-genome sequencing (WGS), and has made it a feasible tool to enhance clinical diagnostic investigations in near real-time<sup>48</sup>. The processes generally involve culture-free parallel sequencing, producing vast quantities of genomic data that require modern computation techniques to assemble the genomic sequence reads as well as performing ensuing analyses that range from identifying the bacterial species or strain, antibiotic resistance mutations in the bacterial genomes, while ensuring the highest possible discriminatory power ever achieved by any technology<sup>49</sup>. Apart from this, WGS of bacteria can identify genes associated with virulence and pathogenicity as well as discover new genetic mechanisms for virulence, pathogenicity and antibiotic resistance<sup>48,50,51</sup>.

The identification and prediction of antibiotic-resistant microorganisms in clinical specimens solely by molecular means in the diagnostic microbiology laboratory is not novel<sup>52</sup>. HTS technologies and computational tools offer unprecedented ability to sequence multitudes of bacterial genomes and enable interpretation of the resultant sequence information in near “real-time”<sup>52</sup>.

WGS represents the pinnacle for bacterial strain characterization and epidemiological analyses. It is rapidly replacing traditional typing methods, antibiotic resistance gene detection and other molecular-based investigations in the near future. HTS technologies are rapidly evolving and their implementation in clinical and public health microbiology laboratories is increasing at a similar pace. These require standardized sample quality control, data interpretation, bioinformatics expertise, and infrastructure. The term ‘bioinformatics’ encompasses the handling and analysis of genomic sequence data, usually with the assistance of computer-based algorithms. Both ‘open source’ and commercially available bioinformatics programs/tools have been specifically developed for use in a clinical setting. However, many of practicing healthcare workers in current practice have limited bioinformatics knowledge<sup>48</sup>.

Furthermore, genome plasticity, as well as pan-genomes that have the ability to influence bacterial resistome can only effectively be investigated using HTS and bioinformatics analyses<sup>48</sup>. Understanding the bacterial genome dynamics is an important step in identifying the forces behind phenotypic antibiotic resistance and therefore allows for effective management of the antibiotic-resistant infections<sup>48</sup>.

HTS and bioinformatics analyses have made it possible to employ comprehensive WGS-based surveillance of colistin

resistance and antimicrobial resistance as a whole by enabling the rapid detection of colistin resistance (i.e. both chromosomal and plasmid-mediated) as well as resistance to other antibiotics; these have also made it possible to map how resistance spreads in a One-Health perspective in a way that was not possible before<sup>53,54</sup>. Furthermore, these have also allowed for rapid re-analysis of large datasets in silico, this has enabled the early detection as well as risk assessment particularly when new genes emerge<sup>53,54</sup>.

## The future direction of HTS

Antibiotic resistance in bacteria is generally a natural phenomenon<sup>55–57</sup> though augmented by human behavior. Therefore, it is imperative to harness the best HTS technologies that sequence DNA at unprecedented speed, to enable previously unimaginable scientific achievements and novel biological applications<sup>53</sup>. Such applications of genomics tools have revolutionized microbial ecological studies and drastically expanded our view on the previously underappreciated microbial world<sup>54</sup> including acquisition and transmission dynamics of antibiotic resistance. Single-Molecule Real-Time (SMRT) sequencing (Pacific Biosciences Inc.) in clinical microbiology has finally been realized at many levels in health care systems in the developing world and relatively only used during isolated scenarios of disease outbreaks in the less developed countries. These developments in HTS must be matched with continued efforts to improve the current bioinformatics analytic pipelines. Applying SMRT while genome sequencing to investigate bacterial colistin resistance would be made possible to predict resistance mutations, resistance mechanisms, trends, and patterns enabling efficient management of the colistin resistance by healthcare providers and pharmaceutical companies.

## Conclusions

It is known that host, bacterial and environmental factors interact collectively to bring about antibiotic resistance. Therefore, HTS should be applied to a wide range of global collections of bacterial whole genomes to identify and predict new antibiotic drug resistance mutations using appropriate computational and bioinformatics algorithms.

Computational algorithms and tools offer the ability to simulate bacterial genomic mutations while also offering possible clues on the mechanisms that may be shaping these mutations. These can as well be utilized to develop therapeutic interventions that may be used to target both the current and future acquired antibiotic drug resistance mutations.

## Data availability

No data are associated with this article

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  56. Wright GD: **Q&A: Antibiotic resistance: where does it come from and what can we do about it?** *BMC Biol.* 2010; **8**(1): 123.  
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  57. Prestinaci F, Pezzotti P, Pantosti A: **Antimicrobial resistance: a global multifaceted phenomenon.** *Pathog Glob Health.* 2015; **109**(7): 309–318.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

# Open Peer Review

Current Peer Review Status:   

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Version 2

Reviewer Report 23 July 2019

<https://doi.org/10.5256/f1000research.21085.r50831>

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**Enrico Lavezzo** 

Department of Molecular Medicine, University of Padova, Padua, Italy

In this short review, Aruhomukama and co-authors discuss about microbial resistance to antibiotics, in particular to colistin, which seems to be the 'last resort' in this era of increasing antibiotic resistance.

They start with a brief report on colistin resistance prevalence in Africa, then they describe the evolution of colistin resistance from a chromosomal feature to a plasmid-related threat, and finally they discuss about high-throughput sequencing and bioinformatics as essential tools for monitoring and contain the rise of antimicrobial genes.

The review is very brief, so I understand that it is not possible to enter in too many details. Nonetheless, there are topics that need to be better discussed and explained, especially in the last section about HTS and bioinformatics, otherwise they are, in my opinion, of no help for the reader.

In the following I list those that are major and minor issues that, to me, the authors should address to improve the quality of the manuscript.

Major points:

- In the abstract, authors mention the search for novel drug targets as one of the topics included in the review, but I found no further information throughout the article. It should be removed from the abstract.
- In the section "The role of high-throughput sequencing technologies and bioinformatics" there is an extremely vague and imprecise definition of what bioinformatics is. I think this is useless: I suppose that all readers know that there are computer-based algorithms for analyzing genomics data and they would rather benefit from at least a brief description of the main steps that bioinformatics tools perform in applications related to clinical settings. And if there is a gap in the ability of clinicians to use these tools, this should be discussed and the reasons explained, also proposing potential solutions.
- The section "The future direction of HTS" starts with a very general description of the potential provided by HTS to study the microbial world. I suppose the authors are referring to metagenomics, even if this word is never mentioned. I think that this application should



- be discussed a little bit more extensively together with its role in the clinical setting.
- In the same section, the authors talk about SMRT sequencing platform (PacBio), but the features of this platform are not explained, and no other platforms are discussed for comparative reasons. Why talking about PacBio here? Which is the advantage/disadvantage with respect to other platforms? In this form, this section is completely detached from the rest of the article.
  - Conclusions: the authors state that HTS should be applied for the identification and prediction of drug resistance genes using appropriate bioinformatics tools. But these tools have never been mentioned in the article. Which tools? Do they need to do?

Moreover, they claim that computational algorithms can be applied for developing therapeutic interventions and target drug resistance mutations. Let's say that this is true, but it is not described in the article so I don't understand how this can be a conclusion of the paper.

Minor issues:

- There are some couples of words that are not separated by a white space throughout the text.  
"Withbacteria", page 4, first columns, line18.  
"phenotypicantibiotic", page 5, first column, line 5 from the bottom.
- Some sentences need to be rephrased for more clarity:  
"These enzymes have in recent...", page 4, second columns, line 3.  
"Also, worth noting is...", page 4, second column, last 3 lines.  
"Furthermore, these have also...", page 5, second column, line 6.
- "Currently studies" à current studies (page 4, first column, halfway)
- "it is rapidly replacing ... in the near future" à it is going to replace?
- "Bioinformatics ... usually with the assistance of computer based algorithms" à why usually?

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Partly

**Are all factual statements correct and adequately supported by citations?**

Partly

**Is the review written in accessible language?**

Partly

**Are the conclusions drawn appropriate in the context of the current research literature?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Bioinformatics, microbiology, molecular biology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 15 July 2019

<https://doi.org/10.5256/f1000research.21085.r48746>

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**Hosam Mamoon Zowawi** 

UQ Centre for Clinical Centre, The University of Queensland, Brisbane, Qld, Australia

**Sharif Hala**

King Abdullah University for Science and Technology, Thuwal, Saudi Arabia

We thank the authors for considering our comments and revising their manuscript, their efforts are clearly reflected in this revised version. We wish them all the best and we have no further comments on this article.

**Is the topic of the review discussed comprehensively in the context of the current literature?**

No

**Are all factual statements correct and adequately supported by citations?**

No

**Is the review written in accessible language?**

No

**Are the conclusions drawn appropriate in the context of the current research literature?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Antimicrobial resistance

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 19 June 2019

<https://doi.org/10.5256/f1000research.21085.r48745>

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**Alexandre Angers-Loustau** 

Joint Research Centre (JRC), European Commission (EC), Ispra, Italy

I would like to thank the authors for their efforts in addressing my concerns with Version 1. I have no further comment on this article.

**Is the topic of the review discussed comprehensively in the context of the current literature?**

No

**Are all factual statements correct and adequately supported by citations?**

No

**Is the review written in accessible language?**

No

**Are the conclusions drawn appropriate in the context of the current research literature?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular biology, bioinformatics

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**Version 1**

Reviewer Report 29 April 2019

<https://doi.org/10.5256/f1000research.19772.r47772>

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**Hosam Mamoon Zowawi** 

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**Sharif Hala**

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The study by Mboowa et al focuses on reviewing the molecular mechanisms underlying the role of Colistin resistance in and the importance of high-throughput sequencing and data analysis in identifying chromosomal and plasmid resistance. The authors explored the spread of colistin resistance genes and gave an example on the spread of *mcr-1* in Africa. They gave examples of the lipid A modification to be linked to two component systems. Moreover, they demonstrated the link between other resistance pathways and colistin where they highlighted examples of mobile colistin elements and tetracycline and carbapenem resistance co-transfer and co-exist. Finally, they described the role of High-throughput sequencing technologies and identified several platforms and methods. Overall, the review is interesting and the data presented in the manuscript is somewhat supporting the conclusion. However, there are a few comments that need to be addressed prior to indexing:

**General comments:**

- The manuscript is written in poor English. Several statements are too long and lack clarity, precision, and completeness. The manuscript has to be rewritten in proper English before submitting it for a detailed revision. As an example "The modifications of the lipid A, which reduce and/or abolish the initial charge-based interaction with the polymyxins in bacteria<sup>13,15,16</sup> and also the addition of either/or the 4-amino-4- deoxy-L-arabinose (L-Ara4N) and the phosphoethanolamine (PEtn) that ultimately form the basis of colistin resistance in bacteria<sup>16</sup>, is mediated by chromosomally encoded genes" \*Very confusing and needs further explanations.
- Authors need to spell out the abbreviation as commonly used. As an example HTS should be NGS (next generation sequencing) in the manuscript.
- In the introduction section, the authors adapted figure 1 of Africa, which talks about countries are not represented in figure 1 and the figure is not matching what is written. Perhaps a different figure could represent the text better.
- The reference quoted need to be checked as I have noticed addition of unnecessary references or references that do not match the text. Example reference 4, 16, 17 are quoted in some places where it's not needed have 3 references and do not match the text.
- The abstract mentions novel mobile colistin resistance plasmid that was not discussed.

**Specific comments:**

- Examples of bacterial type and specific chromosomal mutations are needed, generalization of the resistance pattern could be misleading especially with chromosomal genes. Also, other known chromosomal genes such as *pmrC*, *crdA/B/C*, *dedA*. Finally, as an example a gene such as *eraR* is known to be linked to colistin only in *Pseudomonas* as a response regulator, therefore, the authors are encouraged to mention that different mutations in different organisms can be identified by NGS.
- Repetition of *mcr-1*, *mcr-2* and *mcr-3* sentences several times and not including the rest of the genes all the way to the newly discovered but not published yet *mcr-9* and maybe mention the location of such genes.

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Partly

**Are all factual statements correct and adequately supported by citations?**

Partly

**Is the review written in accessible language?**

No

**Are the conclusions drawn appropriate in the context of the current research literature?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Antimicrobial resistance

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.**

Author Response 14 May 2019

**Dickson Aruhomukama**

We appreciate all the comments made by the reviewers, these helped us to make the manuscript even much better. In the revised manuscript we addressed the comments made as follows;

- General comments – we totally agree with comment (i) in regards to a number of sections being too long and, lacking clarity, precision, and completeness, we have reviewed the entire manuscript, identified sections with these concerns and have endeavored to make these shorter, more clear, precise and, complete. In regards to comment (ii), we agree that HTS and WGS are interchangeably used, however, since we used HTS in the manuscript we have to choose to maintain the use of HTS. Changes were made to address comment (iii), (iv) and, (v) as well.
- Specific comments – (i) we agree that not all chromosomally mediated resistance mechanisms were discussed, however, we ultimately highlight the potential of WGS in the identification of these as well as acquired resistance mechanisms. Comment (ii) was addressed as well.

**Competing Interests:** Non-Financial Competing Interests

Reviewer Report 27 March 2019

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**Alexandre Angers-Loustau**

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In this review, the authors describe the biology behind the evolution of antimicrobial resistance, with a focus on the resistance against colistin, one of the "last-resort" antibiotics. An alarming development was reported in 2016, where a form of resistance was discovered that could be readily transferred to other bacteria. Researchers first discovered this resistance in China, quickly followed by findings of similar resistance patterns in other countries. These discoveries relied in part on the use of high throughput sequencing.

The review is very short and it is understandable that, within these four pages, the authors can't expand these themes in too many details. Still, I have a few minor and a few major comments about the current version of the article.

Minor comments:

- The flow of the different sections of the review is a bit off in my opinion:

a) The section "Colistin resistance trends in Africa" describes the observed burden of colistin resistance in this continent

b) the section "Evolution to plasmid mediated colistin resistance ..." describes the biology and mechanism of colistin resistance, from chromosomal to plasmid-mediated.

c) the section "Pan-drug resistance and characteristics..." describes, generally, the phenomena that gives rise to AMR.

It would make more sense, to me, to rework these sections in the order c), b) then a).

- The sentence "Furthermore, phenomena such as genome plasticity and pan genomes that have the ability to influence bacterial resistome can only effectively be investigated using HTS and bioinformatics analyses." needs to be better explained and referenced. Also, pan genomes are not, in my opinion, "phenomena".
- The section "Evolution to plasmid mediated colistin resistance..." explains the mechanisms of action of chromosome-mediated resistance, then simply lists the genes involved in plasmid-mediated resistance, with no indication about modes of action of these genes, and whether it is different from the ones described previously.
- I noticed at least one incomplete sentence ("Plasmid transfer of the colistin resistance mobile genes, mcr-1, mcr-2, and also mcr-3.")

Major comments:

- The section on Africa needs to be reworked. First, the Figure says "Adapted from Xavier et al. under a CC-BY 4.0 license". I don't know about the license and rights, but I didn't notice any difference with the original figure, so a more appropriate phrasing would be "Reproduced from..." (or, if I missed it, a better indication on how it was adapted).
- Second, I don't see how the figure supports the statement "The limited available literature from African settings has reported the mcr-1 gene mediated colistin resistance to be most prevalent in Africa, largely in South Africa, and this covers the largest portion of Africa according to the global map"; according to the figure, detection in Africa does not look

more prevalent compared to other continents.

- Finally, the text mentions detection in South Africa, Algeria, Rwanda and Uganda, while the maps shows South Africa, Algeria, Tunisia and Egypt.
- The last section, "The role of high-throughput sequencing ..." is very generic, and barely addresses how this applied to colistin resistance, as the title of the review suggests. It contains very general statements about the possibilities of HTS for AMR detection, that have been reviewed in more details in a few recent publications. The authors should take the opportunity of assessing how HTS led to the rapid detection of the spread of the newly identified colistin resistance risk, in a way that was not possible before. Relevant studies should include, among others, the following:

Hasman, H., Hammerum, A.M., Hansen, F., Hendriksen, R.S., Olesen, B., Agersø, Y., Zankari, E., Leekitcharoenphon, P., Stegger, M., Kaas, R.S., et al. (2015). Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat<sup>1</sup>.

Kluytmans-van den Bergh, M.F., Huizinga, P., M. J. Bonten, M. Bos, K. De Bruyne, A. W. Friedrich, J. W. Rossen, P. H. Savelkoul, and J. A. Kluytmans. (2009) Presence of *mcr-1*-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009<sup>2</sup>.

Yu, C. Y., Ang, G. Y., Chong, T. M., Chin, P. S., Ngeow, Y. F., Yin, W. F., & Chan, K. G. (2016). Complete genome sequencing revealed novel genetic contexts of the *mcr-1* gene in *Escherichia coli* strains<sup>3</sup>.

## References

1. Hasman H, Hammerum AM, Hansen F, Hendriksen RS, et al.: Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill.* 2015; **20** (49). [PubMed Abstract](#) | [Publisher Full Text](#)
2. Kluytmans-van den Bergh MF, Huizinga P, Bonten MJ, Bos M, et al.: Presence of *mcr-1*-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. *Euro Surveill.* 2016; **21** (9): 30149 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Donà V, Bernasconi OJ, Pires J, Collaud A, et al.: Heterogeneous Genetic Location of *mcr-1* in Colistin-Resistant *Escherichia coli* Isolates from Humans and Retail Chicken Meat in Switzerland: Emergence of *mcr-1*-Carrying IncK2 Plasmids. *Antimicrob Agents Chemother.* **61** (11). [PubMed Abstract](#) | [Publisher Full Text](#)

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Partly

**Are all factual statements correct and adequately supported by citations?**

Partly

**Is the review written in accessible language?**

Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular biology, bioinformatics

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 14 May 2019

**Dickson Aruhomukama**

We appreciate all the comments made by the reviewer, these helped us to make the manuscript even much better. In the revised manuscript we addressed the comments made as follows;

- Minor comments – (i) we respect the opinion of the reviewer in regards to reworking the sections, however, we noted that this would grossly distort the manuscript and hence choose to maintain the order as is. We agree with the concerns raised in comments (ii), (iii) and, (iv), we addressed each of these as reflected in version 2 of the manuscript.
- Major comments - we agree with the reviewer's comments in regards reworking the section on Africa (i.e. comments i, ii and, iii), these have all been addressed in version 2 of the manuscript. In regards to comment (iv), we reviewed the articles suggested by the reviewer and added a section that briefly describes how HTS leads to the rapid detection of the spread of the newly identified colistin resistance risk. The articles were referenced as well.

**Competing Interests:** Non-Financial Competing Interests

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