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Title	MCR-1 mediated colistin resistance is rare among E. coli clinical isolates obtained from patients in Canadian hospitals (CANWARD 2008-2015)
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Reviewer 1	Dr. David Haldane
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General comments (author response in bold)	A brief description of the types of laboratories would be helpful: were they teaching hospitals or did they include community hospitals or private laboratories. This information might be useful in how to generalize the results. **The laboratories all serve large, tertiary care centres. This is now explicitly stated in the text.** The authors used EUCAST breakpoints. They should indicate that the testing method was compatible with the use of these breakpoints. **The testing method used is consistent with recommendations from the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. This is now stated in the text (and a reference has been added).** One of the primers used for the detection of mcr-1 was designed in house. A reference to the validation of the primer for detection should be given to assure the reader that the primer is sensitive and specific. **We have modified one of the mcr-1 primers so as to fit into a multiplex PCR reaction containing primers for ESBL gene detection. Since there have been very few mcr-1 isolates collected to date, it is difficult to validate the PCR using a large collection of isolates. We have had seven known mcr-1 to date in Canada and all have been positive using our in-house multiplex PCR and have been confirmed by sequencing the amplicon. In addition, we have a synthetic mcr-1 gene which is also positive using the multiplex. We include the synthetic mcr-1 as a positive control in our PCR reaction protocols.** That the study only detected 2 isolates having the mcr-1 gene was reassuring. A graph showing the occurrence of colistin resistant isolates over time might be useful to demonstrate the statement on page 8 that there was "no clear trend towards increasing colistin resistance...". **This information is already presented in Table 1. A graph was not added as this was thought to be redundant. If absolutely required for publication, this could be re-visited.** Since this was a sample of the participating laboratories, were the authors able to give a 95% confidence interval for the incidence? **There was some variability from year to year in the participating centers and the exact number of isolates that were sent to the co-ordinating laboratory. While the data support that colistin resistance (and the mcr-1 gene) are rare among E. coli in Canada, a confidence interval cannot be provided.** In the discussion, the lists of countries might be easier to follow if they were grouped geographically. **This suggestion has been incorporated into the text.** The paper by Mulvey et al reported on an mcr-1 positive isolate in 2011. As that year was not included in this study, the authors could indicate that this isolate was in addition to the ones detected in this study. **The isolate described previously by Mulvey et al. from 2011 was not found as a part of the CANWARD study. As such, it would not be appropriate to include it as a part of the current manuscript dataset. The text does clearly state that the 2 mcr-1 positive isolates in the current study are distinct from those previously reported by Mulvey et al.** On page 10, line 34, why do the authors think that the restriction in testing was unlikely to have missed many isolates? **As indicated in the discussion, most isolates with the mcr-1 gene in other publications have had a colistin MIC of ≥4 µg/mL. Hence, limiting testing to isolates with a colistin MIC of ≥4 µg/mL should capture the majority that are mcr-1 positive.**
Reviewer 2	Prof. Dr. Ed Kuijper
Institution	Department of Medical Microbiology, LUMC, Leiden, The Netherlands
General comments (author response in bold)	MRC-1 mediate colistin resistance has also been found in other members of Enterobacteriaceae family; why have the authors focussed on E. coli? Please include this as an important limitation of the study. **This study focused on E. coli because to date mcr-1 mediated colistin resistance has been most**

	frequently described in this species. The limitations section of the manuscript has been modified to state the following: "Finally, members of the family Enterobacteriaceae other than E. coli were not included in this analysis." Two MRC-1 colistin resistant E. coli isolates were found in blood cultures from patients admitted to the emergency room. This suggests that colistin resistance can be considered as community-acquired. How many of the 5571 investigated isolates derived from patients with an acquired infection outside healthcare facilities? We need at least data from the total number of positive blood cultures with E. coli, taken at emergency rooms of the particapating hospitals to know the prevalence of colistin resistance among E. coli isolates from the "community". **This is addressed under the fourth editorial comment.** Recently, a second form of plasmid mediated colistin resistance has been found; Xavier et al. Eurosurveillance June 2016, Identification of a novel plasmid-mediated colistin resistance gene, mcr-2, in Escherichia coli, Belgium. Please consider to test the remaining 10 phenotypically colistin resistance strains for MRC-2 or just mention it in the discussion. **We have added a sentence for the PCR detection method and reference to the Materials and Methods: Detection of mcr-1 and mcr-2 section "The mcr-2 was detected by PCR using the previously described methods (Xavier et al.)". We have also added to a sentence in the Results "Of the colistin-resistant E. coli isolates, only two tested positive for the mcr-1 gene and the mcr-2 gene was not detected in any of the isolates".** Page 5, line 33. "On an annual basis, each center was asked to submit clinical isolates (consecutive, one per patient/infection site) from blood (100 to 240), respiratory (100 to 150), urine (25 to 100), and wound (25 to 100) infections. The medical centers submitted clinically isolates, as defined by their local site criteria. Please elucidate. Did the authors requested only E. coli? **Isolate submission to the CANWARD study was not restricted to any particular species. However, this study only included E. coli isolates obtained as a part of CANWARD. The text has been modified slightly to clarify this.** Page 6, line 12. Measuring colistin resistance is difficult. Please mention the included controles for colistin resistance. Ref 16 (CLSI) is included as a reference method to test for colistin susceptibility, but we need information whether this method is suitable to recognize MRC1-gen mediated colistin resistant Gram-negatives. The authors have used the cut-off values of EUCAST. The microbiological methods for colistin suspcetibilty testing have been extensively investigated by the CLSI-EUCAST joint Polymyxin Breakpoints Working Group with recommendations published on March 16th, 2016 (www.eucast.org). Please include your comments on this recommedation in the discussion. **The susceptibility testing methods used were consistent with those recommended by CLSI and EUCAST. A reference to the Polymyxin Breakpoints Working Group is now provided.** Page 6, results, line 41. Though the authors mentioned that Enterobacteriacease were tested for colistin resistance, they only report on E. coli isolates. **It is not clear what this comment refers to. The methods section does state that antimicrobials used in the treatment of Enterobacteriaceae were tested against the study isolates, but it does not state that other members of the Enterobacteriaceae were included or tested. No changes have been made.** Page 8, discussion, line 48 , Mulvey et al. Three MCR-1 E. coli isolates have been cultured in another study, including two isolates from ground beef. Please mention their characteristics and similarity with the isolates from this study. Has MLST been applied? Results of the plasmid analysis? **Since the manuscript submission, we have sequenced the genomes of the two mcr-1 positive isolates. We have added a section at the end of the Materials and Methods section to describe this work. From this new data, we were able to generate the MLST types for these two isolates. We have added this information to two sentences in the Results as follows: "The isolate had a colistin MIC of 16 µg/mL and a sequence type of ST648" and "The colistin MIC for this isolate was 4 µg/mL and the sequence type was ST515". In addition, we have added a statement in the Discussion to answer the reviewers question as to the relatedness of the three previously described Canadian mcr-1 isolates. The sentence is as follows "The two MLST types of the isolates identified in this study (ST515 and ST648) were not closely related to the three other previously reported Canadian isolates (Mulvey, personal communication)".** **We are unable to comment on the plasmid type that harbours the mcr-1 in these two isolates as the current whole genome assemblies are detailed enough to definitively determine plasmid types.** Page 9, LIne 22: limitations. Please include the recent reported finding from The Netherlands on MCR-1 positive E.coli, susceptible to colistin due to an IS element in the MCR-1 gene (E.M Terveer et al, ECCMID 2016, Amsterdam). **The limitations section of the paper already states that the mcr-1 gene may be infrequently detected among colistin-susceptible isolates, in the following text: "However, infrequently this gene has been detected among isolates with an MIC of 2 µg/mL."**