

Detection of Colistin Encoding Resistance Genes MCR-1 in Isolates Recovered from Broiler Chickens in Morocco

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Abstract: Plasmid-mediated resistance gene MCR-1 presence in MDR *E. coli* represents a paradigm shift in colistin resistance and poses serious threats in colistin use as last resort drug treatment in animal and human health. Following the first MCR-1 description, researchers around the world published multiple reports about its presence in *Enterobacteriaceae*. In Morocco, among 560 APEC isolates from septicemic broilers thoroughly scrutinized by the disc diffusion method as an initial screening followed by MIC reference technic, 12 strains were suspected as potential carriers of MCR-1 gene. PCR and Sanger sequencing permitted confirmation of MCR-1 presence in three strains giving sequence genes with 100 % homology to MCR-1 gene. Results were concordant with multiple reports about chicken suspicions as a potential reservoir for MCR-1 carrying *E. coli*. Therefore, the use of colistin in animal health must be more controlled and urgent establishment of a national program for antimicrobial resistance surveillance become crucial.

Keywords: MCR-1, Colistin, Avian pathogenic *E. coli*, Antibio-resistance, Morocco.

INTRODUCTION

Colistin was always considered as last resort treatment for multi-drug resistant (MDR) enterobacteriaceae. This situation has changed ever since the description of plasmid-mediated colistin resistance gene *mcr-1* in November 2015 [1]. A veterinary study was conducted in 2012 on poultry to assess the current situation in antibacterial resistance of Avian Pathogenic *E. coli* (APEC) [2]. The broiler sector was targeted because it is the largest provider of animal protein to the country and also the highest consumer of antibacterials especially colistin [3].

Abbreviations:

MCR-1: Mobilized Colistin Resistance type 1

MDR: Multi-Drug Resistant

APEC: Avian Pathogenic *Escherichia coli*

MIC: Minimum Inhibitory Concentration

A total of 560 *E. coli* isolates from septicemic broilers were screened between January 2012 and December 2017, for antibacterial resistance using disc diffusion method under the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Preliminary results showed 26 (4.6 %) resistant isolates with inhibition zones \leq 13 mm by using 50 μ g colistin sulfate discs (Oxoid) [2]. Minimum inhibitory concentration (MIC) method on MH broth reduced the number of colistin-resistant strains to twelve. PCR was performed using a previously described protocol using two sets of primers CLR5-F (5'-CGGTCAGTCCGTTTGTTC-3'), CLR5-R (5'-CTTGTCGGTCTGTAGGG-3') (1) and CLR5-F1 (5'-

ATGATGCAGCATACTTCTGTGTGG-3'), CLR5-R1 (5'-TCAGCGGATGAATGCGGTGC-3') [4]. Only three strains were positive by PCR with amplicons of 256 bp and 794 bp. The analysis of the three sequences (deposited in Genbank nucleotide database under accession numbers MH425549, MH425550 and MK143409) revealed a 100% sequence homology with MCR-1 [1].

This is the first description of MCR-1 carrying *E. coli* isolates from poultry in Morocco and the fourth report (for both animal and human isolates) in Africa after Tunisia [5], South Africa [6] and Egypt [7-9]. Finding only three strains as MCR-1 carriers suggest that other mechanisms of colistin resistance were involved but not included for research in the present study. The most likely mechanisms would be other MCR gene variants (MCR-2 and MCR-3) [10-12]. Additional studies are underway to test the presence of

other resistance genes, but also to characterize the strains and to unravel the involved plasmid groups.

These results highlight that broiler production can be an important reservoir of *mcr-1*-carrying *E. coli* isolates. In addition, colistin extensive consumption in Moroccan broiler production functions as a strong selective pressure [3]. Another concern lies in the fact that broiler litters likely containing live *E. coli* strains are widely used as soil fertilizers which further complicates the epidemiology of MCR-1 underlining the importance of the One Health approach.

In conclusion, we report here the emergence of MCR-1 gene responsible for *E. coli* resistance to colistin in boiler chicken in Morocco. It is urgently needed to reconsider the appropriate use of colistin in veterinary medicine. Such measures will reduce the zoonotic risk that may represent MCR-1 gene carrier bacteria especially among professionals in the poultry industry. This result also suggests the necessity and the urgency for establishing a national program for monitoring antibacterial resistance.

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