Indian J. Anim. Hlth. (2020), 59(1) : 29-32
DOI:10.36062/ijah.59.1.2020.29-32

Review Article

EMERGENCE OF COLISTIN-RESISTANCE- AN UPDATE†

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Colistin, an age old drug which lost attention following introduction of more effective and less toxic drugs, has again been favoured by the clinicians for its supremacy over the modern drugs for treating infections caused by refractory pathogens like carbapenem-resistant Enterobacteriaceae. However, emergence of plasmid-mediated colistin resistance came as a major setback. Animal sector, which had long history of colistin use as growth promoter, particularly in countries like China and Vietnam, has been blamed for emergence and spread of colistin-resistance across the globe. This paper describes various aspects of colistin resistance and its spread across the animal sector.

Key words: Colistin, Food-animals, mcr, Polymixin

Overview

Colistin, a member of polymyxin group, is synthesized from certain strains of gram-positive nitrogen-fixing bacteria, *Paenibacillus polymyxa* (also known as *Bacillus polymyxa*) (Loho and Dharmayanti, 2015). It is a decade-old drug and has been in clinical practices since 1950s. Since 1959 with the advent of colistimethate sodium (CMS), a much less toxic and inactive form of the drug, injectable colistin has become available for treatment of many infectious diseases caused by gram-negative bacteria (GNB) in Japan, Europe and the United States. Further, during 1980s, colistin lost the attraction because of its nephrotoxicity (Kelesidis and Falagas, 2015) and neurotoxicity (Velkov *et al.*, 2018) and gradually moved out of the remedy list with the introduction of aminoglycosides. Of late, its usefulness has been proved in infections caused by some sorts of refractory pathogens like carbapenem resistance Enterobacteriaceae.

Colistin is a polycationic compound mainly effective against the gram negative bacteria. Its electrostatic interaction with the lipid A moiety of the lipopolysaccharide of gram-negative bacteria caused displacement of divalent cations attached to the phosphate groups of the LPS and thereby destabilizing the outer cell membrane (OM). The OM becomes porous leading to the leakage of intracellular contents and finally death of the bacteria (Aghapour *et al.*, 2019).

Colistin resistance

Bacteria employ several mechanisms to develop colistin-resistance which is mainly driven by inappropriate use of the drug. Gram-negative bacteria to become colistin-resistant reduce the overall negative charge of the LPS of the OM to avoid interaction with the positively charged colistin or polymixin. In order to bring such changes, lipid A moiety of LPS is modified by addition of 4-amino-4-deoxy-L-arabinose

†This paper was presented at the National Seminar on *Antimicrobial resistance - A global menace: One health approach* on March 07, 2020 at West Bengal University of Animal and Fishery Sciences, Kolkata, India organised by Indian Journal of Animal Health

(L-Ara4N) or phosphoethanolamine (pEtN). Cationic substitution by L-Ara4N is known an effective way to reduce the overall charge of LPS. Such alteration ultimately reduces the affinity of LPS to colistin (Chen and Groisman, 2013). Further, many GNB are naturally and intrinsically resistant to polymyxin or colistin. Among them important are *Serratia* spp., *Edwardsiella tarda*, *Burkholderia cepacia*, *Proteus* spp., *Providencia* spp., *Morganella morganii* and most of them have their LPS modified with L-Ara4N (Olaitan *et al.*, 2014). Pleiotropic two-component regulatory system- PhoP-PhoQ and PmrA/PmrB - helps the bacteria to survive in the presence of stress by modifying the LPS to evade host defense mechanism and bactericidal agents (Chen and Groisman, 2013). The activation of PhoP-PhoQ or PmrA/PmrB system results in a chain of events which eventually leads to LPS modification and its decreased affinity to colistin (Olaitan *et al.*, 2014). Disruption of *mgrB* gene - a negative regulator of PhoP-PhoQ system- was also reported to cause colistin resistance among *Klebsiella pneumoniae* (Simon *et al.*, 2017). Additional resistance mechanism which the bacteria may employ is increased synthesis of capsular polysaccharides to entrap colistin molecules before they enter the cell (Zhou *et al.*, 2016) and changes in efflux pump takes place.

Lately, a plasmid-mediated resistance gene *mcr-1* that encodes phosphoethanolamine transferases, the enzyme crucial for modification of lipid A- the key step for colistin-resistance- was identified in Enterobacteriaceae (Gao *et al.*, 2016; Wang *et al.*, 2018). This gene has been so far detected among five species – *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Enterobacter cloacae*. More importantly, animal sector, where it was first identified, has been hold accountable for emergence and spread of plasmid mediated colistin-resistance. Apart from human and dogs, wide range of food animals such as cattle, poultry, pigs were reported to have *mcr* carrying bacteria, possibly a consequence of overuse of colistin as growth promoter even in the high income countries (HICs) where it was banned a few years back. Because of presence and carriage of the gene via mobile genetic element, *mcr* mediated resistance may spread rapidly and thus, has been detected in food chain, animal products and even in healthy human microbiome across the globe. Gradually, other alleles/variants of this gene came into the picture *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* (Fukuda *et al.*, 2018).

Colistin-resistance in animals

People Republic of China witnessed the first instance of plasmid-mediated colistin resistance in an *E. coli* strain, SHP45 from pigs (Liu *et al.*, 2016). The *mcr-1* gene was identified in 78 (15%) of 523 samples of raw meat and 166 (21%) of 804 animals during the survey conducted for four years (2011–2014). Later on, the gene was reported in extended spectrum β-lactamase (ESBL)-producing *E. coli* strains along with other multiple resistance traits [resistance to trimethoprim (*dfrA12*), tetracycline {*tet (A)*}, aminoglycoside (*aadA3*, *aph(32)-IA*), phenicol (*cmlA1*), quinolone (*qnrS1*, *oqxA*), lincosamide {*lnu(F)*}, sulphonamide (*sul2*, *sul3*)] from rectal swab of pigs and large areas of the pig slaughterhouses located in the Hoai Duc region of the Hanoi province, Vietnam (Malhotra-Kumar *et al.*, 2016). Soon, *mcr* was reported in APEC from poultry in China (Lima Barbieri *et al.*, 2017). Subsequently, it was reported in *E.* coli isolate from bovine (Palmeira *et al.*, 2018).

A retrospective study conducted with 1611 *E. coli* isolates of chicken origin, collected for a period of five decades, 1974–2014, showed incremental increase of *mcr-1* positive isolates from 2009 to 2014. This was correlated with use of colistin as animal feed additive during this 5-year period. However, the workers also identified 3 *E. coli* strains of 1980 with *mcr-1*. Of late, plasmid-mediated colistin resistance – *mcr-1* – was reported in human clinical isolate from Egypt (Elnahriry *et al.*, 2016). In another retrospective study at Great Romagna Hub Laboratory, Italy, a total of 19,053 isolates belonging to Enterobacteriaceae were

analysed for colistin resistance. Of them, 90 were colistin-resistant. The gene *mcr-1* was detected in 26 *E. coli* with an overall prevalence of 0.14% (Del Bianco *et al.*, 2018).

A new variant, plasmid-mediated *mcr-2* was reported in *E. coli* isolates of porcine and bovine origin (Xavier *et al.*, 2016). Again, another allelic variant *mcr-3* was detected in a porcine *E. coli* with about 45.0% and 47.0% nucleotide sequence identity to *mcr-1* and *mcr-2*, respectively. Other allelic variants were also reported within a short period (Cannatelli *et al.*, 2013, 2014; Borowiak *et al.*, 2017). Newly identified *mcr* gene(s) *mcr-4* and *mcr-5* were also detected from the upper and lower alimentary tracts of pigs and poultry in China. Subsequently, other countries across the world, including India (Ghafur *et al.*, 2019) also reported such plasmid-mediated colistin-resistance among isolates from food animals.

How to contain the spread of colistin-resistance

It is well-known that antimicrobial use is the important driver for AMR and therefore, reducing or optimizing the use of colistin will be the main strategy to reduce its resistance. Many countries reduced the use of colistin as growth promoter or in prophylaxis and passed regulations to eliminate it from the animal feed. Government of India also banned the use of colistin in food animals. Hopefully, if these regulations are implemented properly, the problem of colistin-resistance will die down. Further, researches are also being conducted to explore additional strategies to adopt alternative therapies that can work against colistin-resistant pathogens.

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Received- 07.03.2020, Accepted- 18.05.2020, Published- 01.06.2020
[The article was presented in the National Seminar on *Antimicrobial resistance - A global menace: One health approach* on March 07, 2020 at West Bengal University of Animal & Fishery Sciences, Kolkata, West Bengal organised by Indian Journal of Animal Health]