Aeromonas spp.

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Aeromonas a Gram-negative bacillus, positive for oxidase and catalase tests, a glucose fermenter, and it is resistant to vibriostatic O/129 (2,4-diamino-6,7-diisopropylpteridine). In humans, it can cause intestinal and extra-intestinal infections. It is important in the medical area, mainly in patients with diarrhea, or with infections in the skin and soft tissue; moreover, it can cause bacteremia, which progresses to sepsis, or endocarditis.

Keywords: colistin ; antimicrobial resistance

1. Introduction

Colistin is a lipopeptide antibiotic from the group of polymyxins. It has a cyclic peptide chain that is linked to a fatty acid. Colistin is used in the medical field, since it is an extended-spectrum antimicrobial, and it is used as a last line of treatment in human infections that are caused by Gram-negative bacilli [1]. Until 2016, resistance to colistin was reported in some genera of bacteria intrinsically and contained in the bacterial genophore, until the presence of a gene called *mcr,* present in a plasmid that confers resistance to this antimicrobial, was detected in an *Escherichia coli* strain [2]. After this report, the number of isolates of various origins with *mcr* genes and a colistin resistance phenotype was increased; in addition, it was found that resistance to this molecule could be transferred horizontally [3][4].

The use of colistin as a treatment for infections increased after the appearance of multidrug resistance phenotypes (MDR) in Gram-negative bacilli and the appearance of carbapenemase-producing enterobacteria type KPC (*Klebsiella pneumoniae* carbapenemase) or NDM (New Delhi metallo-β-lactamase), in addition to Gram-negative bacilli classified as XDR (extensively drug resistant) that continue to appear, especially in bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and other Gram-negative bacilli, such as *Aeromonas*, which is also of medical and veterinary importance and is isolated from environmental samples [5][6].

Aeromonas is a Gram-negative bacillus, positive for oxidase and catalase tests, a glucose fermenter, and it is resistant to vibriostatic O/129 (2,4-diamino-6,7-diisopropylpteridine) [7]. In humans, it can cause intestinal and extra-intestinal infections. It is important in the medical area, mainly in patients with diarrhea, or with infections in the skin and soft tissue; moreover, it can cause bacteremia, which progresses to sepsis, or endocarditis [8][9].

The genus *Aeromonas* is widely distributed in diverse ecosystems; however, it is a bacterium native to aquatic systems, hence the largest number of isolates are from water. Isolates have been obtained from drinking water, wastewater, bottled water, seawater, and deep and surface water samples. Food isolates have been obtained from vegetables, fruits, pork, poultry, and beef, as well as seafood and fish. In animals, it is considered a pathogen, especially in fish, in which it can cause furunculosis, ulcers, and hemorrhages, among other diseases. This pathogen has also been isolated from infections in rabbits, dogs, cats, chickens, horses, and crustaceans [8][9].

2. Antimicrobial Resistance in *Aeromonas*

The molecular basis of antimicrobial resistance in *Aeromonas* spp*.,* has been widely studied, but their importance in the hospital area as a cause of outbreaks is not fully established. Likewise, reports on resistance are varied regarding the origin of isolation and the type of antimicrobials tested in vitro [7]. The *Aeromonas* resistance profile has not changed significantly; until now, the mechanism of action of inducible chromosomal β-lactamases and carbapenemase expression has been suggested as being the main resistance mechanism for *Aeromonas* species. Three classes of β-lactamases are recognized in *Aeromonas*; one of class C cephalosporinase, one of class D penicillinase, and one of class B metallo-β-lactamase (MBL) [9][10].

The occurrence of MDR-type *Aeromonas* spp*.,* isolates has been increasing. Different authors have suggested that antimicrobial resistance in the clinical setting is closely related to resistance mechanisms detected in environmental

isolates [11]. In the genus *Aeromonas*, the occurrence of MDR strains is equivalent, due to their origin in aquatic environments, which is attributed to the extensive use of antibiotics in aquaculture. Therefore, this environment becomes an ideal setting for the acquisition of these mechanisms of resistance to antimicrobials and other toxic agents [12].

Colistin Resistance in *Aeromonas*

Since the report in 2016, where it was shown that colistin resistance can be encoded by the *mcr* genes detected within a plasmid, it was determined that these genes are not only in bacterial genophores but can also be present in mobile genetic elements as plasmids [2]. From this report, attention was paid to the search for and detection of these genes in different bacterial genera, mainly those of medical importance, but also isolated from other sources, such as the environment or animals.In *K. pneumoniae*, *P. aeruginosa*, or others, such as *Aeromonas* genus, *mcr* gene variants have been detected, and the reports are increasing [13][14].

Colistin resistance in *Aeromonas* has been reported in several regions of the world, mainly in Europe and Asia. Resistance to this antibiotic has been reported in Latin America in other genera, but not in *Aeromonas*. The detection of colistin resistance is more common in the clinical area; however, colistin-resistant strains of *Aeromonas* have been isolated from other origins that have been detected, from which investigations and reports have emerged in the world. The extensive use of antibiotics in aquaculture and in human treatment has led to an increase in the resistance of this genus to antimicrobial drugs [11][12].

The species *A. dhakensis, A. hydrophila, A. caviae*, and *A. veronii* are considered the main causes of human infections that can cause infection in wounds, diarrheal syndromes, and other clinical presentations [9]. Commonly, the isolates do not present resistance to antimicrobials; however, MDR isolates have still appeared, and in recent years the report of *Aeromonas* isolates from clinical samples and from various sources with resistance to colistin has increased [15]. This resistance has been investigated in *Aeromonas* spp., isolates, by means of disk diffusion test and by minimum inhibitory concentration (MIC), showing the MIC method to be more effective. Induction of colistin resistance in the strains showed an 85% increase after overnight incubation in a tube with *Müller–Hinton* broth and a 50 µL colistin disk. This result allowed the establishment of a phenotypic marker in the *Aeromonas* isolates [16].

In an *A. veronii* isolate from chicken meat, two adjacent genes with colistin resistance markers, called *mcr-3.3* and *mcr-3*-like, were detected in the genophore. The result had 95.2 and 84.19% identity in the nucleotide sequence, when compared to the *mcr-3* gene of an *E. coli* of porcine origin [17].

The evidence of the *mcr* genes in *Aeromonas* was demonstrated by a group of scientists who analyzed a total of 6497 strains that were collected in 13 provinces of China between 2016 and 2017. In these samples, the presence of the *mcr-3* genes was detected by PCR. The *mcr-3* gene was detected in 49 strains only, of which eight strains corresponded to the genus *Aeromonas*, two *A. hydrophila* strains, one with *mcr-3.8* variant, and one with *mcr-3.9* variant, one *A. caviae* with *mcr-3.1* variant, and one *A. media* with *mcr-3.6* variant. Of the four remaining strains, one each were of *A. veronii, A. media,* and *A. caviae*, and one was *Aeromonas* spp., with *mcr-3* without variant. All the strains were grouped into a subclade, after the phylogenetic analysis of the sequences of the *mcr-3* genes detected in the strains [18].

Another group of researchers found four *Aeromonas* isolates with the presence of the *mcr-3* gene through PCR, while the *mcr-1* or *mcr-2* genes were not detected. Each of the four isolates with *mcr-3* genes presented a different variant each; these presented identities in the amino acid chain were of 95 to 98% compared to the original protein MCR-3. These variants of the protein were designated as MCR-3.6 obtained from the *A. allosaccharophila* strain isolated from *Leuciscus idus*, MCR-3.7 for the protein detected in the *A. media* strain isolated from *Meleagris gallopavo*, MCR-3.8 for that detected in the *A. jandaei* strain isolated from a *Cyprinus carpio carp*, and MCR-3.9 for the protein of the *A. hydrophila* strain of *Cyprinus carpio*. The isolate with the *mcr-3.9* gene also contained an additional *mcr-3.8* gene in the MIC test, with colistin showing an MIC ≥ 128 mg/L higher compared to the other isolates [19].

The reports include a new variant of the *mcr-3* gene in *A. caviae*, also detected in *Proteus mirabilis* and *E. coli* that were isolated from a domestic duck. These strains were obtained from sewage samples from free-range ducks, which were raised near a river in the suburban area of Qingdao, Shangdon Province, in China. The presence of the *mcr-3* gene was demonstrated in 1 of 15 samples processed in this study. The result was obtained by detection of the *mcr* gene directly in the sample; the positive sample was seeded in a CHROMagar plate from Biomerieux®, France, to which 2 mg/L of colistin was added. Based on the above, three positive strains were detected for *mcr-3* gene. *A. caviae* 17AC, *P. mirabilis* 17PM, and *E. coli* 17EC strains were identified by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) technology, and by *16S rRNA* gene sequencing [19]. In another study, the prevalence was determined, complemented by a

genetic analysis of the *mcr-3* gene in *Aeromonas* species. These isolates were obtained from human rectal exudates, meat for human consumption, and environmental water samples [20].

The variant *mcr-5* gene of colistin resistance was detected in an *A. hydrophila* strain isolated from a fecal sample from a backyard pig. In this case, the *mcr-5* gene was detected in a plasmid with 7915 base pairs (bp) named pI064-2. Additionally, they analyzed the possibility of transforming the *A. hydrophila* strains susceptible to colistin into a resistant strain [21].

Various mechanisms of resistance to colistin, in addition to the mechanism mediated by *mcr* genes, have been described in some bacteria, including *P. aeruginosa, A. baumannii,* members of the *Enterobacteriaceae* family, such as *E. coli, Salmonella* spp., and *K. pneumoniae* they have an acquired resistance against colistin. However, the possibility of the appearance of strains resistant to this antibiotic should be monitored, due to the presence of mutations, new mechanisms, or adaptations [22].

The protein generated by the *mcr* genes confers resistance to polymyxins; this protein, called MCR, from the inner membrane, adds a molecule of phosphoethanolamine (PEA) to lipid A of lipopolysaccharide (Kdo2-PEA-Lipid A), synthesized by the binding of uridine diphosphate (UDP) and N-acetyl glucosamine (GlcNac) mediated by Lpx proteins (C, D, H, B, K, L, M). The product binds to 2-keto deoxioctanate acid (Kdo2), which is transported by the MsbA protein to the inner membrane, where the PEA molecule is added. The Lpt protein complex (ABCFD/DE) transports the modified LPS that generates resistance to colistin, since the negative charge with which it interacts was modified (Figure 1) [23][24][25].

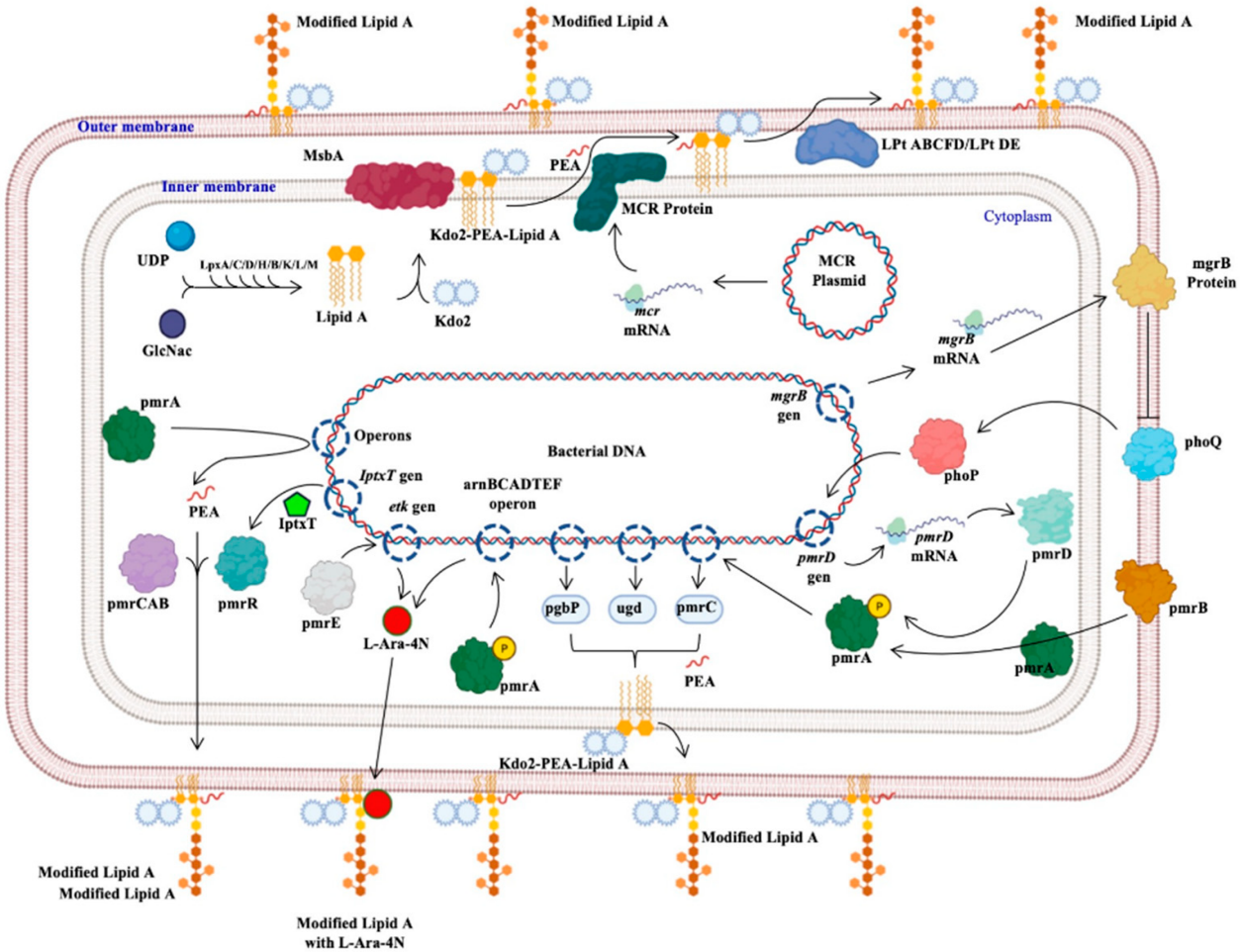

Figure 1. Bacterial mechanisms of resistance to colistin. The different mechanisms of resistance to colistin described in Gram-negative bacilli and other bacteria are presented: the mechanism mediated by the *mcr* genes, those regulated by the phoP and phoQ proteins, others regulated by the pmr proteins or by the *iptxT* and *etk* genes, and operons that encode activation proteins of pmr proteins [22][23][24][25]. Generated from this work.

3. Conclusions

The genus *Aeromonas* is a bacterium widely distributed in the environment. Its presence in various ecosystems and ability to cause infections in animals and humans makes it a bacterium of interest in the study related to the appearance of strains resistant to antimicrobials. The presence of antimicrobial-resistant isolates from the first line of care, and those of

the last alternative, such as colistin, detected in the clinical area, in animals or in the environment, demonstrates that the resistance comes from the exposure of bacteria to antimicrobials in clinical care, but it is also suggested that resistance originates in the environment. In addition, it is important to study *Aeromonas* isolates with colistin resistance from different sources, and also with the detection of the *mcr* genes.

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