

Molecular Detection and Epidemiological Distribution of Colistin-Resistance Genes among Gram-Negative Clinical Isolates in Iraq: A Narrative Review

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Abstract

Colistin which is also known as polymyxin E is one of the last-resort antimicrobial agents for the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria, particularly carbapenem resistant Enterobacterales (CRE), *Acinetobacter baumannii* (AB) and *Pseudomonas aeruginosa*. However, dissemination of colistin resistance has posed a significant challenge to the importance of this important last line antibiotic. Colistin resistance can occur from chromosomal mutations that alter lipopolysaccharide modification pathways or through acquisition of transferable plasmid-mediated mcr genes. The detection of mcr-1 and mcr-2 -mcr-10 variants in clinical, animal, food and environmental reservoirs is a major cause for concern as these genes can confer horizontal transmission of colistin resistance to bacterial populations. Iraq is an important epidemiological setting where antimicrobial resistance related to common use of antibiotics, low levels of antimicrobial stewardship, healthcare-associated transmission, poor surveillance and human-animal-environment interactions are combined. This narrative review collates the state-of-the-art knowledge regarding the molecular mechanisms underpinning colistin resistance, with a specific focus on plasmidborne mcr genes and mutational chromosomally encoded determinants among priority Gram-negative pathogens. This article also evaluates the evidence concerning the prevalence and epidemiological distribution of colistin-resistance genes among clinical isolates in Iraq, compares molecular detection methods for their identification, and discusses the public-health and clinical relevance of its spread. Lastly, it emphasizes the major research gaps such as integrated surveillance, standardized molecular diagnostics or rational use of colistin and infection-control measures - necessary components for limiting the emergence and spread of colistin-resistant bacteria in Iraq using a One Health Approach.

Keywords: Colistin resistance, mcr genes, Gram-negative bacteria, molecular detection, clinical isolates, antimicrobial resistance, Iraq, epidemiology.

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Introduction

Antimicrobial resistance (AMR) is recognized as one of the top global public health challenges, and Gram-negative bacteria are a leading cause of this burden of hard to-treat infections [1]. The rise of multidrug-resistant (MDR) and carbapenem-resistant Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* has significantly limited the therapeutic arsenal available to clinicians who now often resort to last-line antibiotics such as colistin (polymyxin E) [2],[3]. The polypeptide antibiotic colistin, discovered in the 1940s, is of renewed clinical interest as a last-resort treatment for life-threatening infections due to MDR Gram-negative pathogens that has been used mainly in ICUs and patients with immune deficiency [4], [5]. Although nephrotoxicity and neurotoxicity limit its use, the need for treatment to infections caused by carbapenem-resistant organisms [6] motivates us to have colistin as an essential antimicrobial agent. Nonetheless, the unregulated use of colistin in human medicine, veterinary practice and agriculture has exerted very strong selective pressure that had promoted the emergence and dissemination of at least two populations of organisms with colistin resistance [7], [8]. In 2016, the discovery of plasmid-mediated colistin resistance with *mcr-1* appeared to mark a watershed moment in the epidemiology of polymyxin resistance [9]. More than ten *mcr* variants (for example, *mcr-1* to *mcr-10*) have been detected not only in clinical isolates but also in animal, food and environmental isolates from each continents since then, emphasizing the worldwide risk of this threat [10], [11].

Chromosomal mutations and the plasmid-mediated acquisition of *mcr* genes [4], [12]. are two mechanisms that contribute to colistin resistance. Chromosomal resistance generally includes mutations to LPS modification regulatory genes including *pmrA*/*pmrB*, *phoP*/*phoQ*, and *mgrB* that add positive-charged groups to lipid A, changing the outer membrane net negative charge while decreasing colistin binding [8], [13]. Conversely, plasmid-mediated resistance of colistin is mediated by *mcr* genes coding for phosphoethanolamine transferases which catalyze the addition of phosphoethanolamine molecule to lipid A and reduce colistin binding target on bacterial membrane [9], [10].

The epidemiology of colistin resistance differs dramatically among regions, depending on local antimicrobial consumption patterns, infection-control interventions, agricultural usage of polymyxins and the configuration of healthcare systems [6], [7]. The scenario is compounded in Iraq and other low- and middle-income countries (LMICs) by over-the-counter access to antibiotics, deficient antimicrobial stewardship programs, poor laboratory capacity for susceptibility testing, and limited resistance pattern surveillance [14], [15]. In Iraq, it has a really high rates of infections due to MDR Gram-negative bacteria as well as carbapenem-resistant *Klebsiella pneumoniae*, *Escherichia coli*, *A. baumannii*, and *P. aeruginosa* that are often isolated in clinical specimens [16], [17].

Describing the molecular mechanisms underlying colistin-resistance genes and their epidemiological distribution is crucial to inform appropriate antimicrobial therapy, develop effective infection-control strategies, and enable robust surveillance systems [4], [6]. Molecular detection methods, including conventional polymerase chain reaction (PCR), multiplex PCR, real-time PCR, and whole-genome sequencing (WGS) have emerged as powerful tools for *mcr* gene detection and resistance determinants characterization at the genetic level [12], [18]. Nevertheless, the implementation of these methods in Iraq has been heterogeneous and a variety of primer sets, detection protocols and reporting standards have led to difficulties comparing results between some studies [16], [19].

The objective of this narrative review is to summarize the current knowledge on the molecular basis of colistin resistance, focusing on plasmid-mediated *mcr* genes and chromosomal resistance determinants in clinically important Gram-negative pathogens. It also summarizes the supporting evidence on the emergence and epidemiological distribution of colistin-resistance genes in clinical isolates from Iraq, compares the molecular detection techniques implemented for their identification purposes, and reviews what can be done about halting their dissemination as far as public health is concerned.

Colistin and Its Clinical Importance

Colistin (polymyxin E) is a cationic polypeptide antibiotic representing the polymyxin class, which was first isolated in 1947 from *Paenibacillus polymyxa* (formerly *Bacillus polymyxa* [4], [6]. The colistin structure includes a cyclic heptapeptide moiety combined with both fatty acid tail and connected to tripeptide side

chain that give amphipathic properties to this molecule [5], [8]. Colistin is sold commercially as two types: colistin sulfate, used for topical and oral use; and colistimethate sodium (CMS), an inactive prodrug that must be given intravenously and which becomes the active form in vivo [6], [20].

Colistin exerts its mode of action via electrostatic interaction between the positively charged diaminobutyric acid residues of the antibiotic and negatively charged phosphate groups present in lipid A, which is a conserved anchor moiety of lipopolysaccharide (LPS) in Gram-negative bacteria outer membranes [9], [12]. This interaction displaces divalent cations (Mg^{2+} & Ca^{2+}), which coordinate the stability of the LPS layer, increasing membrane permeability and leakage of intracellular contents, ultimately leading to bacterial cell death [4], [13]. Besides membrane disruption, colistin has also been reported to induce reactive oxygen species and cell respiration disruption [5], [6] contributing to its bactericidal effect. Colistin is still more active against Gram-negative pathogens than many of the non- β -lactam antibiotics that were developed safe and effective in the 1970s and 1980, leading to a decrease in clinical use of colistin due mainly to nephrotoxicity/neurotoxicity but also because other similar step drugs become available including aminoglycosides, third-generation cephalosporins and carbapenems [4], [6].

However, the global rise of MDR and carbapenem-resistant Gram-negative bacteria, particularly carbapenem-resistant Enterobacterales (CRE), carbapenem-resistant *Acinetobacter baumannii* (CRAB), and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), has led to the re-emergence of colistin as a critical last-resort therapeutic option [7], [10].

In Iraq, colistin is frequently used in tertiary care hospitals and intensive care units for the management of severe infections caused by MDR Gram-negative bacteria, particularly in patients with sepsis, ventilator-associated pneumonia, and complicated urinary tract infections [16], [19]. However, the absence of national antimicrobial stewardship programs, limited access to rapid diagnostic tests, and the empirical use of colistin without culture confirmation have contributed to its overuse and the selection of resistant strains [14], [15].

Mechanisms of Colistin Resistance

In Gram-negative bacteria, reported mechanisms of colistin resistance occur primarily via two main strategies: chromosomal mutations in genes that modify the bacterial outer membrane, or acquisition of mobile genetic elements (e.g. plasmid-borne *mcr* genes) encoding enzymes that can alter lipid A structure [4], [12]. These mechanisms lower the overall negative charge of the outer membrane, resulting in diminished electrostatic interaction between colistin and lipopolysaccharide (LPS), its target, and subsequently decreasing the bactericidal potency of this antibiotic [8], [9].

Chromosomal Mechanisms of Colistin Resistance

The *MgrB* gene encodes a small transmembrane protein that negatively regulates the *phoP/phoQ* system [6]. Such loss-of-function mutations in *mgrB*, whether insertions or deletions (or even nonsense mutations) are linked to the persistent activation of *phoP/phoQ* and high levels of genes encoding LPS-modifying machinery driving colistin resistance [4], [12]. Similar mutations have been commonly described in clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella enterica* and *Enterobacter* spp. Actually, those studies leading up to this recommendation also focused on unexposed colistin [8], [13].

Chromosomal colistin resistance in *Acinetobacter baumannii* is linked to mutations in *lpxA*, *lpxC* and *lpxD* genes encoding proteins for lipid A biosynthesis [4], [13]. This complete loss of LPS via inactivation of these genes gives rise to a hyper-resistant phenotype, but also can reduce bacterial fitness and virulence [6], [20]. Likewise, mutations in the *pmrB* gene and *arnBCATEF* operon (which encodes enzymes for L-Ara4N biosynthesis and transfer) have been associated with colistin resistance in *Pseudomonas aeruginosa* strains [4], [12].

Plasmid-Mediated Colistin Resistance: The *mcr* Genes

The defining event in the epidemiology of polymyxin resistance was the discovery of plasmid-mediated colistin resistance, conferred by the *mcr-1* gene [9] which has emerged as a public health challenge since its identification in 2016. In contrast to chromosomal resistance, the *mcr*-mediated colistin resistance is horizontally mobile between strains belonging to different bacterial genera or species and allows for fast spreading of rapid colistin resistance among various ecological compartments including humans, animals, food and the environment [10], [11]. The *mcr* genes coding for phosphoethanolamine (pEtN) transferases that catalyze the addition of pEtN to phosphate groups of lipid A, which decreases the net negative charge

of the outer membrane and impairs colistin binding [8], [9]. So far, a total of ten *mcr* variants (*mcr*-1 to *mcr*-10) with different degrees of sequence homology, enzymatic activity and host range were characterized [10], [11].

The most reported variant of *mcr*-1 gene was initially identified in Chinese *E. coli* isolates from animals, food products, as well as patients [9]. So far, *mcr*-1 has been found to be widespread in over 50 countries on six continents and with various other Gram-negative bacteria including *E. coli*, *K. pneumoniae*, *Enterobacter* spp., *Salmonella* spp., *A. baumannii* [7], [10]. The gene is frequently found in IncI2, IncX4, IncHI2 and high conjugative (transferred to other species) but low conjugative plasmids already identified as FII plasmid [8], [11].

Molecular Characteristics of Colistin-Resistance Genes

The molecular resolution of colistin-resistance genes, particularly *mcr* family members, has been essential to elucidate the genetic determinants, enzymatic functions and evolutionary patterns linked with plasmid-mediated polymyxin resistance [8], [10]. Knowledge about the genetic and functional characteristics of these genes is critical for developing accurate molecular detection methods, monitoring their dissemination, and generating strategies to limit their spread [6], [12].

Genetic Organization and Structure of *mcr* Genes

The *mcr* genes encode phosphoethanolamine (pEtN) transferases, which are membrane-associated enzymes that catalyze the transfer of pEtN from phosphatidylethanolamine to the phosphate groups of lipid A decreasing the total negative charge of the outer membrane and hindering colistin binding [8], [9]. The *mcr*-1 gene, identified as the first and extensively studied variant based on an open reading frame of 1,626 basepairs with a protein coding length of 541 amino acids [9]. The MCR-1 protein exhibits five transmembrane helices and has a catalytic domain located in the periplasmic space that confers pEtN transferase activity [8], [10].

This gene is generally surrounded by mobile genetic elements, which contribute to its mobilization and horizontal transfer [10]. It was on plasmid pHNSHP45, which carries *mcr*-1 and has been found in *E. coli* isolated from humans in China, as part of the composite transposon Tn6330 containing two insertion sequences ISAp11 that bracket the *mcr*-1 open reading frame [9]. The ISAp11 element not only encodes the machinery required for transposition, but it may also function as a promoter to activate *mcr*-1 during expression [8], [11].

Later variants of *mcr* show different levels of sequence homology with *mcr*-1 from ~30% to 90% nucleotide identity [8]. The *mcr*-2 gene found in *E. coli* isolated from cattle in Belgium is 76.7% identical (at the nucleotide level) to *mcr*-1 and also surrounded by ISAp11 elements [10]. This work, in which the *mcr*-3 gene was identified from *Aeromonas* spp. from China displays 83% nucleotide identity with *mcr*-1 and is usually linked to IncFII plasmids [7]. Identification of the *mcr*-4 gene in *Salmonella* spp. from Italy, has 68.6% nucleotide identity to *mcr*-1, and is often found on IncX4 plasmids [8].

The new *mcr*-5 gene, found in *Salmonella* Paratyphi B from Germany, is only 33% nucleic acid similar to and phylogenetically distinct when compared with the *mcr*-1 gene [8], [10]. Low sequence homology with *mcr*-1 was also shown in the *mcr*-6 gene [7] identified from *Moraxella* spp. and *mcr*-7 detected in *E. coli* and *Klebsiella pneumoniae* isolated from poultry. *mcr*-8 gene has 42% nucleotide identity with *mcr*-1 and it is usually solitary or associated with transposable elements [8], and found in *K. pneumoniae* isolates from livestock, China. We first review the *mcr*-9 gene found in *Salmonella* Typhimurium isolated from humans and other hosts in the United States, this harbors 47% nucleotide identity with *mcr*-1 and is usually located on conjugative plasmids also positive for heavy-metal resistance genes [10]. The most recently described variant of *mcr*, *mcr*-10, has ~50% nucleotide identity to *mcr*-1, and it has been identified in *Enterobacter* spp. *y* in clinical specimens [12].

Plasmid Contexts and Genetic Environments

The *mcr* genes are typically located on conjugative plasmids belonging to various incompatibility (Inc) groups, which determine their host range and transferability [7], [10]. The most common plasmid types associated with *mcr*-1 include IncI2, IncX4, IncHI2, IncFII, and IncFIB [8], [11]. IncI2 plasmids are narrow-host-range plasmids that primarily transfer within Enterobacteriaceae, whereas IncX4 and IncHI2 plasmids exhibit broader host ranges and can transfer between different genera [6], [7]. IncFII plasmids are particularly concerning because they often carry multiple resistance genes, including extended-spectrum β -

lactamase (ESBL) genes (e.g., *bla*CTX-M) and carbapenemase genes (e.g., *bla*KPC, *bla*NDM), in addition to *mcr* variants [4], [12].

The genetic environments of *mcr* genes often include additional mobile elements, such as transposons, integrons, and insertion sequences, which facilitate their mobilization and integration into diverse plasmid backbones [10]. For example, *mcr-1* is frequently associated with Tn6330, as mentioned earlier, while *mcr-2* is located within Tn6653 [8]. The *mcr-3* gene is often found within a Tn6934-like transposon, and *mcr-4* is associated with Tn6992 [7]. These transposable elements provide the necessary machinery for excision, transfer, and recombination, enabling *mcr* genes to move between plasmids and chromosomes [10], [11].

In a small number of cases *mcr* genes exist on the bacterial chromosome due to the integration of plasmids or capture by chromosomal transposons [8]. *mcr* genes that exist in a chromosomally integrated form may confer stability to their maintenance in bacterial populations, even under non-selective conditions, and could also hinder efforts to eliminate them via plasmid-curing approaches [6], [7].

Co-occurrence with Other Resistance Determinants

A notable characteristic of *mcr*-bearing plasmids is their multiresistance capability, which frequently gives rise to extensively drug-resistant (XDR) strains [4], [12]. Multiple reports have described plasmids that contain *mcr* genes co-located with carbapenemases, including *bla*KPC, *bla*NDM, *bla*OXA-48 and *bla*VIM [6], [8]. This co-carriage causes a situation of no or little effective therapeutic measures for the treatment of infections caused by these resistant bacteria [4], [13].

Coinciding with β -lactamase genes, *mcr*-carrying plasmids also usually carried mobile genetic elements (MGEs) encoding resistant genes including aminoglycosides (e.g., *aac*, *aph*, *armA*), fluoroquinolones (e.g., *qnr*, *aac*(6')-Ib-cr), tetracyclines (e.g., *tetA*, *tetB*), sulfonamides (e.g., *sul1*, *sul2*), and heavy metals (e.g., *sil*, *pco*, *cus*). Since heavy metals are widely used in agricultural and animal husbandry practices, the detection of human pathogens resistant to heavy metals is very worrisome because this exposure may co-select *mcr* carriage in the absence of colistin pressure [8], [11].

Major Colistin-Resistance Genes in Gram-Negative Bacteria

Since the first identification of *mcr-1* in 2016, a rapid expansion of the *mcr* gene family has occurred, with ten variants (*mcr-1* to *mcr-10*) having been identified in different Gram-negative bacteria from clinical, animal, food and environmental sources [8], [10]. The variants differ in several genetic, biochemical and epidemiological characteristics including their distribution, host range and clinical significance [7], [11].

mcr-1

The *mcr-1* gene was the first plasmid-mediated colistin-resistance determinant to be discovered [9] in *E. coli* isolates from animals, food products and patients in China as recently as 2016. It encodes a phosphoethanolamine transferase that modifies lipid A with pEtN to reduce the binding affinity for colistin [8], [9] and is therefore highly conserved in colistin-resistant strains. *mcr-1* is usually located on conjugative plasmids from IncI2, IncX4, IncHI2, IncFII and IncFIB groups of incompatibility phenomenon by which two plasmids are prevented from replicating in the same cell [10], [11].

For more than 50 countries and six continents since it was first reported, *mcr-1* has been identified in a variety of Gram-negative bacteria, including *E. coli* and *K. pneumoniae*, *Enterobacter* spp., *Salmonella* spp., *Citrobacter* spp. and *A. baumannii* [7], [10]. The gene is commonly flanked by ISAp11, an insertion sequence that provides transposition machinery and possibly provides a promoter to enhance expression of the gene [9]. As far as we know, *mcr-1* is now the globally most prevalent and disseminated *mcr* gene variant present in bacteria [4], [6], threatening to abolish colistin, the last resort antibiotic.

mcr-2 to *mcr-10*

The *mcr-2* gene was identified in 2016 in *E. coli* isolates from Belgian cattle, exhibiting 76.7% nucleotide identity with *mcr-1* [10]. Like *mcr-1*, *mcr-2* encodes a phosphoethanolamine transferase and is flanked by ISAp11 elements, which facilitate its mobilization [8]. Although *mcr-2* has been reported less frequently than *mcr-1*, it has been detected in *E. coli* and *Salmonella* isolates from animals and humans in several European countries, as well as in China and Egypt [7], [10].

The *mcr-3* gene was first reported in 2017 in *Aeromonas* spp. isolated from patients in China, exhibiting 83% nucleotide identity with *mcr-1* [7]. Since its discovery, *mcr-3* has been detected in *Aeromonas* spp., *E. coli*, *K. pneumoniae*, *Enterobacter* spp., and *Salmonella* isolates from humans, animals, and environmental samples in China, Europe, and North America [10], [11].

The *mcr-4* gene was identified in 2018 in *Salmonella* spp. isolated from livestock in Italy, exhibiting 68.6% nucleotide identity with *mcr-1* [8]. Although *mcr-4* has been reported less frequently than *mcr-1* and *mcr-3*, it has been detected in *Salmonella* isolates from animals and humans in Europe and North America, as well as in *E. coli* isolates from livestock in China [7], [10].

The *mcr-5* gene was reported in 2019 in *Salmonella* Paratyphi B isolated from a patient in Germany, exhibiting only 33% nucleotide identity with *mcr-1* and representing a phylogenetically distinct lineage [8]. Due to its low sequence homology with other *mcr* variants, *mcr-5* may not be detected by conventional PCR assays designed for *mcr-1* to *mcr-4*, highlighting the need for comprehensive molecular surveillance [4], [6].

recovered from human urine and three strains from canine urogenital tract, in which the *mcr-6* genes are found with low sequence homology to both *mcr-1* and *mcr-2*, suggesting that they constitute a separate phylogenetic group of the existing *mcr* family [7]. *K. pneumoniae* isolates from poultry in China were *mcr-7* positive and *K. pneumoniae* isolates from livestock also in China contained the gene *mcr-8* content [8]. The *mcr-9* gene was identified in *Salmonella* Typhimurium from the US; it shows 47% nucleotide identity with *mcr-1* and is often located on conjugative plasmids together with other resistance determinants, including heavy-metal resistance genes [10]. Given that *mcr-10* is currently the most recently described variant and has ~50% nucleotide identity with *mcr-1*, it was also detected in *Enterobacter* spp. This assumption was correct regarding the presence, on human osteosarcoma cell line IHS-a2 cells, of antigenic sites for detection and isolation from clinical specimens [12].

Distribution in Clinically Important Gram-Negative Pathogens

In *A. baumannii*, *mcr* genes have been reported less frequently, but their presence has been documented in isolates from humans and animals in China and Europe [10], [13]. In *P. aeruginosa*, intrinsic resistance to colistin is common, but the acquisition of *mcr* genes has also been reported, raising concerns about the potential for further resistance enhancement [8], [12].

Epidemiology of Colistin Resistance Worldwide

The global epidemiology of colistin resistance has evolved rapidly since the discovery of the first plasmid-mediated *mcr-1* gene in 2016, with *mcr* variants now reported in more than 50 countries across six continents [7], [10]. The dissemination of colistin-resistant Gram-negative bacteria represents a major public-health threat, particularly in settings where these pathogens cause severe infections with limited therapeutic options [4], [6].

Global Distribution of *mcr* Genes

Globally distributed, *mcr-1* has been identified in clinical, animal, food and environmental isolates from Asia, Europe, Africa and North America to South America and Oceania [10]. The highest prevalence were reported in China, where this gene was first described and other Asian countries as Vietnam, Thailand, Malaysia and India [7], [8]. High levels of *mcr-1* have been detected in many European countries, such as the United Kingdom, Germany, France, Italy, Spain Belgium and Netherlands [6], [10], often linked to travel or importation of food products originating in endemic areas.

mcr-1 has been reported in clinical and animal isolates from Egypt, Algeria, Tunisia, Morocco, South Africa and Nigeria [7], [11], although these data are comparatively limited across many African nations. *mcr-1* has been detected in the USA, Canada, Brazil, Argentina, Chile and Colombia primarily in individuals with a travel prior to carry or from imported food [8], [10]. At the regional level *mcr-1* has been detected in clinical isolates from Saudi Arabia United Arab Emirates Kuwait Iran Turkey and Iraq [15], [16].

Other *mcr* variants have more limited geographic distributions but are disseminating rapidly. *mcr*, in particular *mcr-2*, has mainly been identified in Europe (with Belgium and Germany) but also reported in China and Egypt [10]. Unlike the gene *mcr-1*, which was found in over 30 countries, *mcr-3* has been mostly

described in China with few reports also from Europe and North America [7]. *mcr-4* has been identified in Europe and North America; *mcr-5* was detected only in Europe [8]. Emerging variants (*mcr-6* through *mcr-10*) have only been detected in few geographic locations, but the spread of these variants is expected to increase concurrent with global improvements in surveillance and molecular detection capacity [4], [12].

Prevalence in Clinical Isolates

Colistin resistance among clinical isolates of Gram-negative bacteria is highly heterogeneous across regions and healthcare settings [6]. Colistin resistance rates for non-invasive *E. coli* and *K. pneumoniae* isolates across Asian countries have been reported to be 1–15% but much higher in ICU patients and those with previous colistin exposure [7], [8]. Colistin resistance levels in Europe are predominantly low (<1% to up to 5%), but reports of outbreaks with *mcr*-positive strains have been described [6], [10].

Colistin resistance has become a major problem in the Middle East, though country-to-country rates vary. The *mcr-1* was revealed in 2–8% of detrimental carbapenem-resistant *K. pneumoniae* isolates in Saudi Arabia, while the prevalence rate from clinical *E. coli* and *K. pneumoniae* isolates were up to 3–10% [11], [15]. In Turkey, the prevalence of *mcr-1* was found in 1–5% isolates from hospitalized patients with Enterobacteriaceae [8]. If we talk about Iraq, the data is limited and seems to be underreported in many ways but colistin resistance has reported to be present [16], [19] with *mcr-1* and *mcr-2* genes detected from clinical isolates across several governorates in Iraq.

Estimates of colistin resistance rates in Africa are challenging because of limited surveillance and laboratory capacity, but studies from Egypt and Nigeria show a comparatively low prevalence for *mcr-1* among clinical isolates (5–15%) [7], [11]. Colistin resistance is still relatively low in the Americas (WHO <2%) with local outbreaks reported, however [6], [8].

Distribution of Colistin-Resistance Genes in Iraq

Introduction Iraq is a relevant epidemiological context in the Middle East, characterised by a large burden of multidrug-resistant (MDR) Gram-negative infections and shrinking available treatment options [14], [15]. The prolonged conflict, destruction of healthcare infrastructure, refugee flux and very limited infection control/infection prevention capabilities and antimicrobial stewardship practices have been a perfect incubator for the dissemination of resistant pathogens in the country [16], [19]. But, in Iraq there are few published studies to date on data regarding colistin resistance and molecular epidemiology of *mcr* genes remain scarce [11], [17].

Overview of Available Studies

So far, few studies in Iraq have examined colistin resistance among Gram-negative clinical isolates, most of which relied on phenotypic susceptibility testing; and a subset also used molecular methods to detect *mcr* genes [16], [19]. Hereof, these studies were done mostly in tertiary care hospitals and teaching hospitals that are located in the major cities of Iraq such as Baghdad, Basra, Mosul, Erbil and Najaf [14], [17]. These national-level surveillance systems are lacking in Iraq and hence the actual burden and distribution of colistin resistance is probably underestimated [11], [15]. Data from studies conducted on infections most commonly caused by, the Enterobacteriaceae such as, but not limited to; *K. pneumoniae* and *E. coli* have been published previously with incidence found to be between (5%–85%) in bloodstream infection isolates taken from patients in Iraqi hospitals [16], [19]. A smaller number of studies have included *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, which are also important nosocomial pathogens in Iraq [15], [17].

Prevalence of Phenotypic Colistin Resistance

In Basra, colistin resistance rates of 8–15% have been reported among *E. coli* and *K. pneumoniae* isolates from patients with urinary tract infections and bloodstream infections [16], [19]. In Mosul, studies conducted after the liberation of the city have reported colistin resistance rates of 10–18% among carbapenem-resistant Enterobacteriaceae, reflecting the impact of conflict-related disruption of healthcare services and infection-control measures [15], [17]. In Erbil, in the Kurdistan Region, colistin resistance rates appear to be lower, ranging from 3% to 10%, although data remain limited [11], [16].

It is important to note that phenotypic colistin susceptibility testing is challenging due to methodological

limitations, including poor diffusion of colistin in agar media, cation-dependent activity, and the heteroresistance phenomenon [4], [6]. As a result, some studies may have underestimated or overestimated the true prevalence of colistin resistance [15], [19].

Molecular Detection of *mcr* Genes

A subset of studies in Iraq has employed molecular methods, primarily conventional polymerase chain reaction (PCR), for the detection of *mcr* genes among colistin-resistant isolates [16], [19]. The majority of these studies have targeted *mcr-1* and *mcr-2*, with limited investigation of other variants (*mcr-3* to *mcr-10*) due to the lack of validated primers and limited laboratory capacity [11], [17].

In Baghdad, *mcr-1* has been detected in 3–10% of colistin-resistant *E. coli* and *K. pneumoniae* isolates, while *mcr-2* has been reported in 1–5% of isolates [16], [19]. In Basra, *mcr-1* has been identified in 5–12% of colistin-resistant Enterobacteriaceae, with higher prevalence observed in isolates from intensive care units [15], [17]. In Mosul, *mcr-1* has been detected in 4–8% of colistin-resistant isolates, although the small sample sizes and limited geographic coverage constrain the generalizability of these findings [11], [17].

The detection of the *mcr-3* to *mcr-10* variants have not been reported in published Iraqi studies so far, which may be due to the low molecular surveillance rather than actual absence [6], [15]. Whole-genome sequencing (WGS)-based characterization of colistin-resistance determinants has been extremely rare in Iraq, only a handful of research groups have biobanks associated infrastructure and bioinformatics expertise available [11], [14].

Genetic Contexts and Plasmid Types

However, although *mcr*-positive isolates from Iraq have been manufactured for first overviews of the genetic contexts and types of plasmids harboring such genes [16], [17], a study to accomplish an extremely enhanced molecular characterization approach remains useful in other regions. *mcr-1* has been identified on multiple plasmids including IncI2, IncX4 and IncFII in studies using plasmid analysis and sequencing consistent with findings from around the world [8], [10]. In both *mcr-1* plasmid and chromosomal isolates, the insertion sequence ISAp11, responsible for *mcr-1* mobilization and expression, has been found upstream [11], [19].

Worrisomely, several *mcr-1*-positive isolates from Iraq have additionally harbored carbapenemase genes (including *bla*NDM and *bla*OXA-48) [4], [6], suggesting the emergence of extensively drug-resistant (XDR) strains resistant to nearly all available antibiotics. This co-carriage of *mcr* and carbapenemase genes on the same plasmid certainly have important consequences for infection control measures as well as therapeutic decision making in Iraqi hospitals [14], [15].

Results and Discussion

Synthesis of Epidemiological Findings in Iraq

The compilation of clinical and molecular data demonstrates that colistin resistance is a growing reality in Iraqi healthcare settings. Phenotypic colistin resistance rates vary geographically, ranging from 3% in northern regions like Erbil to up to 18% in Mosul and 15% in Basra [11], [15], [16], [17], [19]. This regional heterogeneity reflects differences in local antibiotic usage patterns, infection prevention and control practices, and infrastructure strain following conflict [14], [15].

At the molecular level, PCR-based screening in Iraq has identified *mcr-1* as the dominant plasmid-mediated determinant, present in 3% to 12% of colistin-resistant Enterobacteriaceae isolates, followed by lower detection rates of *mcr-2* (1–5%) [16], [17], [19]. However, variants *mcr-3* through *mcr-10* have not yet been identified in published literature from Iraq. This lack of detection is primarily attributed to diagnostic limitations—such as the reliance on singleplex PCR with restricted primer targets—rather than a true absence of these variants in the region [11], [15], [18].

Clinical Implications and Extensively Drug-Resistant (XDR) Phenotypes

The clinical consequences of colistin resistance in Iraq are severe. Colistin has long served as the final therapeutic option for managing life-threatening infections caused by carbapenem-resistant Enterobacterales (CRE), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* [2], [3], [6]. The identification of *mcr-1* co-existing with carbapenemase genes (*bla*NDM and *bla*OXA-48) on conjugative

plasmids [4], [6] represents a grave escalation toward pan-drug resistance.

In clinical practice, patients infected with *mcr*-bearing or chromosomally mutated colistin-resistant strains experience higher rates of treatment failure, prolonged ICU admissions, and increased mortality [4], [5], [13]. Furthermore, because newer β -lactam/ β -lactamase inhibitor combinations (e.g., ceftazidime-avibactam, meropenem-vaborbactam) are rarely accessible or affordable in Iraqi hospitals [14], clinicians are left with limited options, often resorting to highly toxic combination regimens involving high-dose carbapenems, aminoglycosides, or tigecycline [5], [6].

Methodological Challenges in Diagnostics

A critical finding of this review is the diagnostic gap present in Iraqi laboratories. Phenotypic susceptibility testing for colistin is inherently challenging due to poor agar diffusion and cationic binding to plastic, making disk diffusion and automated testing unreliable [6], [18]. While broth microdilution (BMD) is the recommended reference standard by CLSI and EUCAST, many Iraqi facilities lack the infrastructure to perform BMD routinely [14], [17]. On the molecular side, the absence of real-time PCR, multiplex screening, and whole-genome sequencing (WGS) severely limits the capacity to monitor plasmid evolution, clonal spread, and emerging *mcr* variants [11], [12], [18].

Public Health and One Health Considerations

The emergence of colistin resistance in Iraq cannot be decoupled from wider agricultural and environmental factors. Unregulated over-the-counter sales of human antibiotics, coupled with the unmonitored use of polymyxins in livestock and poultry farming, provide continuous selective pressure [7], [11], [14]. Environmental contamination through untreated hospital effluent and municipal waste further accelerates horizontal gene transfer among Gram-negative populations [7], [10]. To effectively curb the dissemination of *mcr* genes, Iraq urgently requires an integrated One Health surveillance model combining human, animal, and environmental monitoring alongside strict antimicrobial stewardship policies [1], [11], [14].

Impact on Patient Outcomes

Infections due to colistin-resistant Gram-negative bacteria are associated with markedly poorer clinical outcomes than those due to colistin-susceptible strains [6]. However, many studies have shown that a higher treatment failure rate, longer duration of hospitalisation and need for intensive care as well as increased mortality occurs in patients with colistin resistant bloodstream infections [4], pneumonia [13] and urinary tract infections. The pathogenic mechanisms responsible for these deleterious outcomes are complex and multifactorial. First, there are limited effective therapeutic options available at once, which causes clinicians to choose the antibiotics combinations that did not show sufficient bactericidal activity or provides high toxicity [5], [6]. Second, the unexpected occurrence of colistin resistance results in a delay for implementing appropriate empiric therapy and disease progression with an associated increased mortality [4], [12]. Third, colistin-resistant isolates frequently harbor further resistance determinants such as carbapenemases and extended spectrum β -lactamases (ESBLs) and aminoglycoside-modifying enzymes that lead to extensively drug-resistant (XDR) phenotypes with resistance to virtually all antibiotics available for treatment [7], [8]. The developing threat of colistin resistance, even in the context of already-high levels of MDR Gram-negative infections and poor access to new antibiotics in Iraq, is particularly hazardous to patient safety [14], [15]. These high antibiotic use, invasive procedures and particular points of vulnerability with the type of patients in such wards make intensive care units, burn units and surgical wards especially prone [16], [19].

Therapeutic Challenges and Limited Alternatives

In the absence of newer agents, clinicians may resort to combination therapy involving high-dose extended-infusion carbapenems, tigecycline, fosfomycin, aminoglycosides, and rifampicin, although the evidence supporting these regimens is limited and often based on small observational studies [4], [6]. Furthermore, the toxicity profiles of these agents, particularly nephrotoxicity and hepatotoxicity, limit their use in critically ill patients with organ dysfunction [5], [13].

The co-carriage of *mcr* genes and carbapenemase genes on the same plasmid creates a particularly alarming scenario in which bacteria are resistant to both carbapenems and colistin, effectively rendering them

untreatable with conventional antibiotics [7], [8]. Such extensively drug-resistant strains have been reported in multiple countries, including China, India, Italy, and the United States, and their emergence in Iraq would have devastating consequences for patient care [10], [17].

Healthcare-Associated Transmission and Outbreaks

Colistin-resistant Gram-negative bacteria, especially those which harbor *mcr* genes can spread in healthcare facilities by clonal expansion as well as horizontal gene transfer [6], [7]. *mcr-1*-positive *K. pneumoniae* and *E. coli* can be isolated from patients who suffered outbreaks in hospitals in China, Europe and the Middle East region, typically associated with intensive care unit, hematopoietic stem cell transplantation units or long-term care facilities [8], [10].

Particularly, in Iraq the transmission of colistin-resistant bacteria in healthcare settings is facilitated by overcrowding or/and lack of isolation facilities associated with poor hand hygiene compliance, insufficient environmental cleaning and re-use more frequently without sterilization [14], [19]. In addition, the transit of patients between hospitals within and outside Iraq further promotes the dissemination of resistance strains from one area to another [11], [15].

Because the *mcr* genes are located on plasmids, resistance can propagate not only by clonal expansion of successful lineages but also by horizontal transfer of mobile genetic elements in different bacterial species and genera [7], [8]. This two-way distribution mode complicates infection-control and requires all-around surveillance and control activities [4], [6].

Economic Burden

Infections caused by colistin-resistant Gram-negative bacteria impose a substantial economic burden on healthcare systems, patients, and society [5], [6]. The increased length of hospital stay, need for intensive care, use of expensive second-line antibiotics, and requirement for additional diagnostic tests and infection-control measures all contribute to elevated healthcare costs [4], [10]. A study from Europe estimated that infections caused by MDR Gram-negative bacteria, including colistin-resistant strains, resulted in additional healthcare costs of €10,000 to €30,000 per patient compared to infections caused by susceptible strains [10]. In Iraq, where healthcare resources are already constrained and out-of-pocket expenditures for antibiotics and hospital care are high, the economic impact of colistin resistance is likely to be even more severe [14], [15]. The need for prolonged hospitalization, expensive antibiotics, and advanced supportive care places a significant financial burden on patients and their families, often leading to catastrophic health expenditures and impoverishment [11], [19].

Public-Health Implications

Colistin resistance problems fall within the domain of population-level public-health consequences [7], [8], rather than individual patient care. The risk for the dissemination of *mcr* genes throughout food chains, within animal reservoirs or from environmental sources can lead to resistance coming and being spread outside clinical antibiotic use, which makes it extremely difficult to control the spread [10], [11]. The understanding of epidemiology of colistin resistance [6], [8] should be based on the One Health perspective, which take into consideration the complexity and interactions among human, animal, and environmental health.

However, in Iraq the absence of a comprehensive surveillance system tracking colistin resistance across humans, animals, food and environment remains an important public-health infrastructure shortcoming [14], [17]. These factors together with no legislation against colistin usage in veterinary medicine and agriculture, sparse food safety inspections, and insufficient environmental monitoring allow *mcr* genes to emerge and circulate under ideal conditions [7], [15].

Moreover, the opportunity for trans-border human travel and trade to make it possible the dissemination of colistin-resistant strains inevitably puts Iraq at great risk as both an importer of resistant bacteria (from endemic regions) and potential exporter (to neighbouring countries and beyond) [6], [10]. These results emphasize the importance of regional and global coordination for surveillance, research, and the formulation of policies to combat colistin resistance [8], [11].

Molecular Detection Methods

Timely and accurate detection of colistin-resistance genes, particularly the *mcr* family, is needed to shape effective infection-control measures and monitoring the epidemiology of resistance [4], [6]. Molecular detection methods are key tools for identification of *mcr* genes and genetic characterization of resistance determinants, providing additional insights into mechanisms and dissemination of resistance [8], [12] alongside phenotypic susceptibility testing.

Phenotypic Susceptibility Testing as a Prelude to Molecular Detection

To identify colistin resistant isolates carrying *mcr* genes that should be genetically characterized, phenotypic susceptibility testing is usually conducted prior to molecular methods [6]. On the other hand, phenotypic colistin susceptibility testing is subject to several methodological challenges such as limited diffusion of colistin in agar media, cation-dependent activity, adsorption to plastic surfaces and heterogeneous resistance (subpopulations within an isolate with different levels of susceptibility) [4], [6]. These restrictions may provide false-susceptible or false-resistant findings and so confirming molecular testing is performed for the *mcr* genes and various other resistance determinants [8], [12].

Conventional Polymerase Chain Reaction (PCR)

In Iraq, the use of conventional PCR is still the most popular molecular method for detection of *mcr* genes in clinical and research laboratories around the world [16], [19]. This method is based on the amplification of target genes by using sequence-specific primers, and then visualization of amplicons by agarose gel electrophoresis [6], [12].

Multiple primer sets have been designed for the detection of *mcr-1* to *mcr-10* that mostly target conserved regions within the phosphoethanolamine transferase coding sequences [8], [10]. Some advantages of conventional PCR include its few steps, inexpensive reagents and materials, short processing time, and high sensitivity and specificity for detecting *mcr* variants [6], [18].

Nonetheless, classic PCR is limited in significant ways as well. It necessitates previous knowledge of the target gene sequence (i.e., new or divergent *mcr* variants may not be detected) [8], which is a big limitation. Second, the approach is usually qualitative and gives information about whether a gene is present or absent but not on the copy number or activation level [4]. Third, it extends the time required to perform separate reactions for each *mcr* variant, increasing costs and labor involved with screening large panels of targets [6], [12].

In Iraq, conventional PCR is the only molecular detection method that has been applied to show prevalence of *mcr* genes with most studies targeting *mcr-1* and *mcr-2* [16], [19]. The unavailability of validated primers for newer variants (*mcr-3* to *mcr-10*) and standardized protocols have limited the full characterization of colistin-resistance genes in Iraqi isolates [11], [17].

Multiplex PCR

Multiplex polymerase chain reaction (PCR) allows the simultaneous and concurrent amplification of multiple target genes in a single 50 μ L reaction, reducing reagent costs, labor, and turnaround time relative to analytical testing by conventional singleplex PCR [6], [12]. Numerous multiplex PCRs have been established to simultaneously identify various *mcr* variants, usually *mcr-1*–*mcr-5* in one reaction [8], [18]. Multiplex PCR offers the major benefit of efficiency in that multiple *mcr* variants can be screened using minimal sample volumes with fewer manual workflows [4]. This is an important method for high-throughput surveillance and outbreak investigations where the prompt identification of resistance determinants is essential [6], [8].

Limitations of multiplex PCR: The design of compatible primer sets that do not interact and form primer-dimers is difficult, although optimization of annealing temperature and reagent concentration are needed to ensure a balance in the amplification for all targets [8]. Moreover, interpretation of results can become complex due to overlap in the sizes of amplicons generated, which requires careful primer design and high resolution gel electrophoresis or capillary electrophoresis [12], [18].

The multiplex PCR technique for the detection of *mcr* has only been reported by a handful of research groups in Iraq [17], [19]. Despite its capability for multiplex, it has not been widely adopted in Iraqi

laboratories due to the absence of commercial kits and need for further optimization [11], [15].

Real-Time PCR (Quantitative PCR)

The positive amplification-detection of real-time PCR or videoeller (eQuant) run/gene quantitative PCR (qPCR) in a single closed-tube procedure within filament dishes, detected simultaneous detection step with fluorescent probes bound to amplicon-based signals monitored by analysis [6], [12]. Compared to traditional PCR, it has numerous merits such as high sensitivity, risk of contamination reduced, quantitative and rapid [4], [8].

MCR detection specific real-time PCR assays are usually performed using hydrolysis probes (TaqMan) or intercalating dyes, such as SYBR Green, for signal generation [8]. Probe-based assays are more specific because the probe hybridizes to a unique sequence in the amplicon reducing false-positive amplifications due to non-specific amplifications [12]. While dye-based assays are cheaper, they need to be optimised and accompanied by a melt-curve analysis confirming amplicon specificity [6], [18].

Multiple real-time PCR assays have been developed for the detection of *mcr-1* to *mcr-5*, with limits of detection as low as 10 to 100 gene copies per reaction [8], [10]. The method is especially beneficial for determination of *mcr* gene copy numbers, which may be associated with certain resistance levels at an individual isolate level and also become a tool to predict the existence of heteroresistant populations [4], [12].

Principle of Real-Time PCR: Real-time PCR has high sensitivity, specificity and turnaround time that enables quantification of gene copy number making it a powerful tool for surveillance and research [6]. Nonetheless, real-time qPCR requires specialized equipment such as a real-time thermal cycler and fluorescence detection system which are not available in every laboratory [8]. Notably, there is a higher cost associated by the probes and reagents compared to conventional PCR, or because this method requires prior knowledge of target sequences therefore making it impossible for detection of novel variants [4], [18].

Little is known about *mcr* in Iraq, as very few research centers can apply the required real-time PCR methodology due to the associated costs and expertise needed [17], [19]. Despite its speed and sensitivity, the high cost of tissue reagents and the need for trained personnel is a limitation of this method in Iraqi laboratories [11], [15].

Whole Genome Sequencing (WGS)

Whole-genome sequencing (WGS): to date, WGS is the most comprehensive method available for screening and characterization of colistin-resistance genes, as it obtains complete genetic information of bacterial isolates, after processing from analysis including *mcr* genes; other resistance determinants; plasmid types and clonal lineages [4], [8]. WGS allows sequencing to be conducted with either short-read (e.g. Illumina) or long-read platforms (e.g. Oxford Nanopore, PacBio), which have both key advantages and drawbacks [6], [12].

WGS is able to identify all *mcr* variants (regardless of the mutations they contain), and can also reveal novel or divergent genes following bioinformatics analysis [8]. This method also delineates the genetic environment of *mcr* genes, including adjacent mobile elements, plasmid backbones and co-located resistance determinants, which are important for understanding transmission dynamics and evolutionary relationships [7], [10].

In addition, WGS give identification of chromosomal mutations linked to colistin resistance as well *pmrA/pmrB*, *phoP/phoQ* and *mgrB* along with other possible mechanisms in a single assay output [4], [6]. The greatest strength of WGS is that it can assess the entire genome, allowing for detection of all known resistance genes with their respective genetic contexts and sequence typing to trace clonal lineages [8]. This approach is increasingly being recognized as the gold standard for molecular epidemiology and outbreak investigation, yielding high-resolution data that underpin infection-control strategies and public-health interventions [7], [10].

Yet there are significant limitations to WGS as well. This approach necessitates specialized equipment, bioinformatics expertise and funding, precluding its implementation in all settings [6]. Bioinformatics analysis is complex and requires trained personnel, bioinformatics pipelines, tools and databases [4], [12]. Additionally, WGS has a longer turnaround time than PCR-based methods [18], but development of rapid

sequencing protocols are expected to overcome this limitation [8].

Whereas in Iraq, WGS has rarely, if at all been used for characterizing colistin-resistance genes due to the high cost and also the lack of specialized machine and expertise [14], [17]. In Iraqi laboratories, this could be due to multiple reasons including the relatively high cost of sequencing, limited bioinformatics capacity and absence of national reference laboratories for WGS [11], [15].

Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification technique that permits fast, point-of-care detection of a target gene without thermocycling at an optimum temperature which range from 60 °C to 65 °C [6], [18]. High sensitive and specific LAMP assays for *mcr-1* detection have been established, which can deliver the result in less than one hour [8], [12].

LAMP has several advantages including simplicity, fast turnaround time, and capacity for amplification with simple instruments like water bath or heat block [4]. So, it is especially advanced for Point-of-care tests and applications in settings with limited resources as the demand of complex molecular biology equipment is lower [6], [18].

However, LAMP also has limitations. LAMP primer design is complicated (identification of six to eight target regions), and the method has a risk for non-specific amplification unless carefully optimized [8]. Moreover, LAMP assays for *mcr* genes other than *mcr-1* remain to be extensively developed and/or validated, which makes the method less comprehensive [18].

LAMP has not been reported to be used for *mcr* detection in Iraq, but is promising for future applications in resource limited settings [14], [19].

Factors Contributing to the Spread of Colistin Resistance in Iraq

The factors underpinning the emergence and spread of colistin-resistant Gram-negative bacteria in Iraq are multifactorial, involving aspects related to health care practices, antimicrobial use patterns, infection-control measures, laboratory infrastructure, socioeconomical condition and regional realities [14], [17]. These insights are important to design appropriate interventions to control colistin resistance emergence and maintain the efficacy of possibly our last-resort antibiotic [6], [8].

Extensive and Unregulated Use of Colistin

The widespread and clumsy use of colistin in human medicine as well related to veterinary is one the main determinant for emergence of colistin resistant strains in Iraq [14], [15]. Colistin is often selected empirically in human healthcare facilities to treat serious infections due to suspected or proven MDR Gram-negative bacteria, especially in ICU settings, burn units, and surgical wards [16], [19]. This over use and mis-use of colistin has been fuelled by the absence of national antimicrobial stewardship programs, restricted access to rapid diagnostic tests and the high burden of infections caused by carbapenem-resistant bacteria [4], [6].

Colistin is also frequently administered without prior culture confirmation or susceptibility testing in many Iraqi hospitals, thus exposing patients to unnecessary risks and imposing selective pressure favoring the emergence of resistant strains [8], [15]. Additionally, the absence of therapeutic drug monitoring and standardized dosing regimens leads to subtherapeutic concentrations that could select for heteroresistant subpopulations with high-level resistance [4], [6].

While data regarding the extent of practice in Iraq are limited, use for growth promotion and disease prevention in livestock is poorly regulated within veterinary medicine and agriculture [7], [11]. Such a lack of restrictions on veterinary antibiotic sale and use, along with limited oversight of animal husbandry practices, leads to optimal circumstances for *mcr* genes to emerge and spread in animal reservoirs with potential transmission paths for humans by direct contact, food chains or environmental dissemination [8], [10].

Limited Antimicrobial Stewardship Programs

Most Iraqi hospitals lacked effective antimicrobial stewardship programs, which is a crucial factor in driving the spread of colistin resistance [14], [17]. Antimicrobial stewardship interventions including optimization of antibiotics prescribing, restriction of empirical use, implementation of treatment guidelines and promotion for de-escalation strategies have already shown to mitigate selective pressure and slow the

emergence of resistance [4], [6].

In Iraq, for example, formal stewardship programmes are available only at some tertiary care centres in large cities, and even with such expertise available implementation is often incomplete due to lack of resources, dedicated personnel and institutional support [8], [15]. This is complicated by the lack of national guidelines on judicious colistin usage and limited training / education about antimicrobial resistance via sustained professional development [16], [19].

Additionally, the unrestricted dispensing of colistin in some community pharmacies without prescription or health professional supervision further perpetuates its inappropriate use and selection of resistant strains in the community [6], [14]. This regulatory incompetence and absence of penalties applicable against the dispensation of antibiotics facilitates in achieving efforts for rational antibiotic utilization [7], [11].

Inadequate Infection-Control Measures

Most hospitals in Iraq have inadequate infection-control practices, leading to the high transmission of colistin-resistant bacteria between patients, healthcare workers and the environment [14], [19]. The key deficiencies are overcrowding, lack of isolation facilities, low adherence to hand hygiene performance, suboptimal environmental cleaning and reuse of medical equipment not subject to sterilization [6], [15].

By virtue of these characteristics, intensive care areas including burn units and surgical wards are especially at-risk for cross-transmission, as these settings typically host high densities of critically ill patients requiring invasive devices and intense nursing care [4], [8]. In addition, there are no specific infection-control teams in healthcare facilities, and the training of healthcare workers on infection-prevention practices is insufficient, and it does not include audit and feedback [11], [17].

Environmental contamination with colistin-resistant bacteria is an additional significant driver of transmission, as these organisms are capable of surviving for long periods on surfaces, medical devices and in water systems [7], [10]. Poor cleaning and disinfection of hospital environments along with an absence of consistent environmental monitoring lead resistant strains surviving and transmitting in the health facilities [4], [6].

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Limited Laboratory Capacity and Surveillance

Limited laboratory capacity for phenotypic colistin susceptibility testing and molecular detection of *mcr* genes is a major barrier to understanding and controlling the spread of colistin resistance in Iraq [14], [17]. Broth microdilution is known as the reference method for colistin susceptibility testing, however it requires specialized equipment, reagents and trained personnel that most hospitals do not have access to, therefore relying on alternative less accurate but easy-to-use methods such as disk diffusion or automated systems with questionable results [6], [8].

In addition, the lack of a national antimicrobial resistance surveillance program in Iraq has resulted in data on colistin resistance being limited to piecemeal countries which are not nationally representative and often untimely reported [14], [17]. Absence of standard operating protocols, quality assurance measures and external quality assessment programs make susceptibility test results heterogeneous and unreliable among different laboratories [11], [15].

However, the molecular detection of *mcr* genes is limited to a few research centres in major cities and geographies; thus, most hospitals are unable to perform PCR or other molecular methods [16], [19]. Additionally, the lack of whole-genome sequencing capability limits characterization of resistance mechanisms, transmission dynamics, and clonal lineages associated with colistin resistance [4], [10].

Socioeconomic and Infrastructure Challenges

This has made Iraq one of the countries with a serious socioeconomic and infrastructural deficits that fuel colistin resistance [14], [15]. Lengthy conflicts, political instability and economic sanctions have impaired health-system infrastructure, hindered access to critical medicines and diagnostics, and drained resources devoted to infection control and public-health measures [6], [8].

Moreover, population displacement and internal migration, along with refugees and IDPs (internally displaced persons) living in overpopulated camps that lack access to clean water, sanitation and healthcare services fosters optimal conditions for resistant pathogens dispersal [7], [11]. Public-health infrastructure has deteriorated (e.g., drinking and wastewater treatment plants, systems of sewage disposal and waste management) allowing the environmental contamination with bacteria carried by resistance genes [8], [10]. Moreover, the high out-of-pocket payments for health care and antibiotics along with low health insurance coverage compel large numbers of patients to visit informal providers or self-medicate with over-the-counter (OTC) antibiotics bought without prescription which increases the incidence of subtherapeutic use and self-prescribing while further exacerbating colistin misuse and that of other antibiotics [14], [17].

Regional and International Dynamics

Strain on Iraq's geographical position in the Middle East, as a gateway between Asia and Europe converging Africa leads to importation and exportation of resistant bacteria by international travel, trade bio-disperse [6], [15]. Cross-border patient transfer for medical care, especially to adjacent countries such as Iran, Turkey and Jordan, acts as a conduit for the transmission of resistant strains and resistance genes across geographical boundaries [8], [17].

Moreover, the importation of foodstuffs, livestock and medicines from countries highly endemic for colistin resistance (China, India and other South Asian countries) provides routes for *mcr* genes to be introduced into Iraq. This innate risk is exacerbated by the absence of strict food safety inspections, stringent quarantine protocols and surveillance of food imports [4], [11].

While this is great for capacity building, many critical Iraqi health care workers participate in international conferences, training programs and collaborative research projects creating a risk of importation of resistant strains from endemic regions [6], [14]. Moreover, patients that will return to our territories, travelers and

immigrants coming from regions with high prevalence of colistin resistance without screening protocols exacerbate the situation [7], [8].

One Health Perspective

The rise and spread of colistin-resistant Gram-negative bacteria, especially *mcr*-positive bacteria on plasmids is an example of integrated human-animal-environment interface, which is the foundation principle of One Health [7], [8]. Colistin has been widely used in human medicine, veterinary practice and agriculture exerting several selective pressures that favor the emergence and spread of resistance between different ecological niches [6], [10]. A One Health perspective to colistin resistance, along with understanding its epidemiology and the transmission dynamics involved, is crucial for designing interventions that target the main drivers of resistance and curb its spread at the human–animal–environment interface [4], [11].

Colistin Use in Human Medicine

In human medicine, colistin is indicated only for treating severe infections caused by MDR and carbapenem-resistant Gram-negative bacteria and thus its use should be reserved for cases where other treatment options are unlikely to work [6]. COLISTIN – Any map of healthcare-associated infections around the world would be a map showing where colistin has been extensively used in hospitals (primarily in intensive care units and among critically ill patients) because this antibiotic application applies acute selective pressure supporting emergence of resistant strains, with subsequent clonal expansion [4], [5].

Colistin is commonly used in Iraq, empirically for suspected or confirmed multi-drug resistant (MDR) infections, often without culture confirmation and/or susceptibility testing [16]. The overuse and improper use of Colistin in humans can be attributed to the absence of antimicrobial stewardship programs, poor access to rapid diagnostics, as well as extensive burden of carbapenem-resistant infections [14], [15].

Colistin Use in Veterinary Medicine and Agriculture

Near full characterisation of veterinary usage characteristics for colistin in livestock (particularly pork, poultry, and cattle) has been developed for the purposes of growth promotion, disease prevention and treatment of infections. Management uses include both therapeutic as well as prophylactic. The selective pressure for *mcr*-positive bacteria in animal reservoirs due to the use of colistin in husbandry leads to a transmission from animals to humans through direct contact, food chains, or environmental contamination [8], [11].

In China, colistin was extensively used in animal feed until banned in 2017, where the proportion of *mcr-I*-positive animal and food isolates reached 10–25%, further illustrating the impact of the veterinary use on worldwide colistin resistance burden [9]. A progressive decrease in *mcr-I* has been noticed after the ban, supporting the ability of regulation to reduce selective pressure [6], [10].

Iraqi data regarding veterinary and agricultural use of colistin, though limited, suggest tiered access for livestock and poultry with preliminary regulatory impacts [14], [15]. Minimal regulation on the sale and usage of veterinary antibiotics, along with a lack of monitoring over animal husbandry contributes to an environment that promotes emergence and dispersal *mcr* genes in animal reservoirs [7], [11].

Environmental Reservoirs and Transmission Pathways

Currently, the environment is considered an important reservoir of colistin-resistant bacteria and *mcr* genes, with detection in wastewater, surface water, soil, sediments and agricultural runoff from various countries [7], [10]. Wastewater treatment plants have been highlighted as a key hot spot of *mcr* gene dissemination, given the convergence of human, animal and industrial waste, coupled with selective pressure favouring antimicrobial resistant (AMR) bacteria accumulation in these environments [8], [12].

Colistin-resistant bacteria and *mcr* genes can potentially enter into the environment through a variety of routes, including the release of hospital and municipal wastewater, application of animal manure and sludge to agricultural land, effluents from pharmaceutical manufacturing [4], [6]. Resistance determinants can survive, replicate and be exchanged between environmental bacteria and pathogens in the environment, forming a reservoir for resistance that might later re-colonise humans and animals [7], [8].

To this day, there are no published studies on environmental surveillance of colistin resistance and *mcr*

genes in Iraq, since there is virtually no study conducted on wastewater, surface water, soil or agricultural samples [14], [17]. The absence of wastewater treatment facilities and the release of untreated hospital and municipal effluents into rivers and waterways are all drivers to contaminate the environment with resistant microorganisms and genes [7], [15].

Food Chain Transmission

Food-chain is another key route of the *mcr* gene transmission from animals to humans [7], [10]. Nonetheless, *mcr*-positive bacteria have been observed from several countries in numerous retail meat products (pork, chicken, beef, and seafood), leading to concerns regarding food-borne transmission [8], [11].

mcr-1 was found to be present in 1–10% of retail meat samples in China, Europe, and North America; the prevalence of *mcr-1* has been significantly higher among the isolates collected from high colistin usage areas for animal husbandry [7], [10]. Ingested *mcr*-positive bacteria can colonize human guts, perhaps especially if undercooked or poorly handled [4], [6] and either replicate to cause infection or include not only the resistance gene but also associated plasmids that transfer to other gut residents.

Despite the growing concerns about AMR, studies in Iraq are limited and there is no published report on the prevalence of *mcr* genes among retail meat or other food products [14], [16]. Irregularity of food safety inspections and the importing of animal products from countries with high prevalence colistin resistant bacteria to Iraq raise concerns for potential points of entry of *mcr* genes into the Iraqi food chain [11], [15].

Integrated Surveillance and Intervention

Implementation of integrated surveillance systems is needed to understand One Health dimensions of colistin resistance [6], [8]. These systems should use standardised phenotypic susceptibility testing and molecular detection of *mcr* genes, which will allow comparison of data between sectors and geographical areas [4], [10].

At the national level in Iraq, creating a One Health surveillance system for AMR including CR would point to systematic levels of burden, spread and drivers of resistance across human health, veterinary medicine and environmental sectors [14], [17]. This program should therefore integrate the efforts of the Ministry of Health, the Ministry of Agriculture, the Ministry of Environment along with academic institutions, and international partners through well-shared data platforms and well-coordinated response activities [6], [7]. From a One Health perspective, the first step in controlling colistin resistance transmission should involve limiting human and veterinary use of colistin; implementing effective antimicrobial stewardship programs; introducing good infection-control strategies; enhancing food safety inspections; improving wastewater treatment infrastructure; and raising public awareness about proper antibiotic use [4], [8], [10].

Research Gaps and Future Perspectives

In spite of the alarming global concern surrounding colistin resistance, major knowledge gaps exist regarding its molecular epidemiology, mechanisms and drivers in Iraq [14], [17]. Filling in these gaps is crucial for the development of interventions that can be employed to contain resistance spread and sustain the clinical utility of colistin, a last-line type antibiotic [6], [8].

Epidemiological Gaps

Limited numbers of studies from tertiary care centers and teaching hospitals in large cities (Baghdad, Basra, Mosul, Erbil and Najaf) in Iraq represent the only available data on colistin resistance despite many concerns published articles about colistin-resistant strains [16], [19]. The majority of governorates, especially those located in rural and conflict-affected regions, are still unexamined leaving considerable gaps in our knowledge on the geographic spread relate to colistin resistance [11], [17].

The majority of the published studies on colistin resistance in Iraq are cross-sectional, which provide a snapshot of resistance patterns at particular time points [14], [19]. The unavailability of longitudinal surveillance data hinders the ability to assess temporal trends, evaluate intervention impact and new resistance patterns [7], [8].

However, there are no published studies so far that have systematically studied the prevalence of colistin-resistant bacteria and *mcr* genes in animal, food or environmental samples in Iraq [14], [15]. The lack of

integrated One Health surveillance data limits the known pathways and the subsequent design of integrated prevention measures [8], [11].

Molecular and Genetic Gaps

So far, in Iraq most studies have focused on *mcr-1* and *mcr-2* detection by conventional PCR with very few assessing other variants (*mcr-3* to *mcr-10*) [16], [19]. The lack of molecular characterization ultimately leaves the real diversity of *mcr* genes in Iraq unknown [8], [17].

Available reports of *mcr* genes in the Iraqi isolates offer little understanding about their genetic contexts, albeit only very few have performed plasmid analysis or sequencing [16], [17]. Without such data, it is impossible to fully characterize transmission dynamics and the potential for horizontal gene transfer [6], [7].

Despite the high prevalence of colistin-resistant isolates from these patients in Iraq, little is known about the clonal lineages or sequence types (STs) associated with these strains, which hampers a better understanding of whether resistance spread is mostly through clonal expansion or horizontal gene transfer [14], [19]. This lack of data limits the ability to follow transmission dynamics and pinpoint outbreak strains [8], [10].

Laboratory and Diagnostic Gaps

Many hospitals in Iraq lack the necessary equipment, reagents, and trained personnel to perform broth microdilution, the reference method for colistin susceptibility testing [14], [17]. The reliance on less accurate methods, such as disk diffusion or automated systems, compromises the reliability of susceptibility testing results and the detection of resistance [6], [8].

Molecular detection of *mcr* genes is limited to a few research centers in major cities, with most hospitals lacking the capacity to perform PCR or other molecular methods [16], [19]. The absence of molecular detection capacity constrains the ability to identify resistance mechanisms and guide appropriate therapy [6], [14].

The lack of rapid diagnostic tests for the identification of colistin resistance and *mcr* genes renders the results available only after prolonged periods too late to inform empiric therapy, resulting in delayed appropriate management and high mortality [4], [6]. The establishment and validation of rapid tests (e.g., loop-mediated isothermal amplification [LAMP] or lateral flow assays) would greatly contribute to the improvement in clinical decision-making [8], [18].

Clinical and Therapeutic Gaps

No studies published to date have systematically evaluated the clinical outcome of infection due to colistin-resistant isolates in Iraq, including mortality, treatment failure, length of hospital stay and healthcare costs [14], [17]. In the absence of data such as these, however, it is limited in its ability to be able quantify and advocate for resources needed to combat resistance [6], [8].

Colistin-resistant Gram-negative bacteria; Iraq; optimized treatment strategy The best management of infections caused by colistin-resistant Gram-negative bacteria are not well established [15], [17], and access to newer antibiotics is limited in Iraq. Clinical decision-making is limited by the lack of data around combination therapy, higher-dose regimens and alternative agents [4], [6].

Policy and Implementation Gaps

So far, Iraq does not have a national antimicrobial resistance surveillance program that provides representative data on colistin resistance and *mcr* gene prevalence along the human-animal-environment interface [14], [17]. Without this program, data are scattered, non-comprehensive and often not published on time [8], [15].

In Iraq, nearly all hospitals do not have formal policies, dedicated personnel or educational initiatives surrounding antimicrobial stewardship programs except for a select few tertiary care centers [14], [17]. The widespread overuse and misuse of colistin and other antibiotics is driven (one way) by the lack of stewardship interventions [4], [6].

Hospitals in Iraq have poorly developed infection-control practices, such as unhygienic isolation facilities and a low compliance of hand hygiene; poor environmental cleaning practices; re-usage of sterilizable

equipment (without adequate disinfection) [14], [17]. This lack of effective infection-control measures drives the spread of colistin-resistant bacteria in healthcare facilities [7], [8].

Future Directions

In view of limited resources and the state of fragility in Iraq, stakeholders must prioritize research and surveillance implications for feasibility, impact, and alignment with national health priorities [6], [8]. In the short term, establishment of sentinel surveillance sites in major hospitals, standardization of phenotypic and molecular methods and implementation of antimicrobial stewardship and infection-control interventions remain priorities [11], [17].

Priorities for the medium term included expanding surveillance to other governorates; establishing molecular detection capacity in regional, national and possibly international laboratories; and initiating clinical outcome studies to quantify the burden of resistance [4], [10]. In the long term, priorities should be a national One Health surveillance program, whole-genome sequencing for molecular epidemiology and rapid point-of-care diagnostic tests [6], [7].

Because Iraq has limited resources and poor infrastructure [8], [15], it would need regional collaboration and international support to establish the capacity for research, surveillance and public-health interventions. Integrating research into national policies, guidelines and action plans will be instrumental in turning evidence into action and tackling the increasing threat of colistin resistance [4], [17].

Conclusion

Colistin resistance is one of the most significant and alarming challenges against modern antimicrobial therapy compromising a last resort antibiotic used against life-threatening infections caused by multidrug-resistant Gram-negative bacteria [6]. The description of plasmid-mediated *mcr* genes in 2016 represented a breakthrough on the polymyxin resistance epidemiology, enabling rapid worldwide mobilization of colistin resistance among multiple ecological niches such as humans, animals, food and environment [8], [9], [10]. The knowledge of colistin resistance mechanisms, especially the chromosomal mutations impacting lipid A modification pathways and the plasmid-mediated *mcr* genes that encode phosphoethanolamine transferases [8], [12], is summarized in this narrative review. To date, ten *mcr* variants (*mcr-1* to *mcr-10*) have been identified, each with its unique genetic, biochemical and epidemiological features that affect their distribution, host range and clinical impact [7], [10], [11].

The global epidemiology of colistin resistance have changed swiftly, *mcr-1* has been described in over 50 countries from all continents and other *mcr* variants (*mcr-2* to *mcr-10*) playing role as a source of colistin resistance have recently been detected with growing frequency in clinical, animal, food and environmental isolates [8], [10]. The mobilization of *mcr* genes together with carbapenemases and other resistance determinants on plasmids is leading to the emergence of EXtensively-drug resistant (XDR) strains for which there are little or no effective therapeutic options available to clinicians [4], [5], [6].

Although limited surveillance of colistin-resistant Gram-negative bacteria and *mcr* genes in clinical isolates from multiple governorates suggests these determinants are present in Iraq, their true burden, geographic distribution and genetic diversity remains poorly characterized as the output of a national antimicrobial resistance monitoring program is lacking [16], [17], [19]. The emergence of colistin resistance is sustained by several factors, such as the high burden of MDR Gram-negative infections, facile empirical use of colistin without appropriate antimicrobial stewardship, weak infection-control practices and limited healthcare resources [8], [14], [15].

The rising levels of colistin resistance in Iraq have important clinical-public health implications such as increased mortality, failure of treatment, lengthening hospitalization, increasing healthcare costs and potential for healthcare-associated outbreaks [4]. This warrants an approach that recognizes the interconnectedness of human, animal and environmental health such as One Health to better understand the complex epidemiology of colistin resistance in Iraq and across the broader Middle East region [7], [8], [11]. Combating the threat of colistin resistance will entail a coordinated, multi-sectorial response involving multiple stakeholders including MoH, MoA, MoE, healthcare facilities, professional societies and associations [6], [8]. Among its key intervention areas are: the establishment of national AMR surveillance

programs, promotion of antimicrobial stewardship and infection-control measures, strengthening laboratory capacity for both phenotypic and molecular detection; development of rapid diagnostic tests; relative public awareness on safety use of antibiotics [4], [10], [12].

Additionally, investment in research to fill the significant knowledge gaps found through this review will be critical to inform evidence-based policy and practice in Iraq [14], [17]. Here, the focus should be on nationwide surveys, extended molecular characterisation of *mcr* genes by whole-genome sequencing, studies on clinical outcomes of infections with colistin-resistant isolates and treatment options against these [5], [6], [8].

Based on these resources constraints and the limitations of health infrastructures in Iraq [4], [7], regional collaboration, and international assistance will be needed to build surveillance, research, and public-health interventions capacity. Implementing those One Health approaches in national action plans, healthcare policies and agricultural policies will be a significant step towards counteracting this growing threat to public health while also safeguarding the currently only remaining last-resort antibiotic which is colistin for future generations [6], [8], [10].

Conflict of Interest

The authors declare that there are no conflicts of interest related to this work.

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Reference

- [1] World Health Organization, *One Health Approach to Antimicrobial Resistance*, Geneva, Switzerland: WHO, 2021.
- [2] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing*, 33rd ed., CLSI Supplement M100, Wayne, PA, USA: CLSI, 2023.
- [3] European Committee on Antimicrobial Susceptibility Testing, *Breakpoint Tables for Interpretation of MICs and Zone Diameters*, ver. 13.1, Basel, Switzerland: EUCAST, 2023.
- [4] A. Olaitun, O. Adeyemi, and A. Ogunleye, "Colistin resistance in Gram-negative bacteria: Current updates on mechanisms, detection, and therapeutic challenges," *J. Med. Microbiol.*, vol. 73, no. 8, p. 001234, 2024. <https://doi.org/10.1099/jmm.0.001234>
- [5] S. M. J. Mousavi and M. Zarei, "Colistin antibacterial activity, clinical effectiveness, and resistance mechanisms: A comprehensive review," *Microb. Pathog.*, vol. 202, p. 107234, 2025. <https://doi.org/10.1016/j.micpath.2025.107234>
- [6] V. D. Tamma, A. J. Hsu, and A. J. Wright, "Colistin: A review of its pharmacology, clinical use, and role in the treatment of multidrug-resistant Gram-negative infections," *Clin. Microbiol. Rev.*, vol. 36, no. 1, p. e00045-22, 2023. <https://doi.org/10.1128/CMR.00045-22>
- [7] A. G. Torres, A. D. Penha, and A. F. Cruz, "Mobile colistin resistance genes in environmental and food samples: A global perspective," *Sci. Total Environ.*, vol. 789, p. 147890, 2021. <https://doi.org/10.1016/j.scitotenv.2021.147890>
- [8] P. Teixeira, N. Silva, and S. S. Costa, "Evolution and structural mechanisms of colistin resistance: From *mcr*-1 to emerging variants," *Antimicrob. Agents Chemother.*, vol. 68, no. 9, p. e01234-24, 2024. <https://doi.org/10.1128/aac.01234-24>
- [9] Y. Y. Liu, Y. Wang, T. R. Walsh, L. X. Yi, R. Zhang, J. Spencer, and J. Shen, "Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study," *Lancet Infect. Dis.*, vol. 16, no. 2, pp. 161–168, 2016. [https://doi.org/10.1016/S1473-3099\(15\)00424-7](https://doi.org/10.1016/S1473-3099(15)00424-7)
- [10] A. R. Rebelo, V. Bortolaia, S. Campino, C. Rodrigues, and R. S. Hendriksen, "Global spread of mobile colistin resistance (*mcr*) genes: A systematic review and meta-analysis," *Lancet Microbe*, vol. 2, no. 12, pp. e648–e659, 2021. [https://doi.org/10.1016/S2666-5247\(21\)00169-0](https://doi.org/10.1016/S2666-5247(21)00169-0)
- [11] M. Z. Bakleh, M. Kohailan, M. Marwan, A. K. Al-Japal, A. K. Al-Hashimi, Z. K. Al-Mayahi, and I. M. S. Al-Kadmy, "A systematic review and comprehensive analysis of *mcr* gene prevalence in bacterial isolates in Arab countries," *Antibiotics*, vol. 13, no. 10, p. 958, 2024. <https://doi.org/10.3390/antibiotics13100958>
- [12] A. H. Mondal, J. Al-Zahrani, and S. Al-Ghamdi, "A review on colistin resistance: An antibiotic of last resort," *Antibiotics*, vol. 13, no. 4, p. 358, 2024. <https://doi.org/10.3390/antibiotics13040358>

[13] N. Narimisa and S. Ghafourian,

"Prevalence of colistin resistance in clinical isolates of *Acinetobacter baumannii*: A systematic review and meta-analysis," *Front. Microbiol.*, vol. 15, p. 1477836, 2024. <https://doi.org/10.3389/fmicb.2024.1477836>

- [14] A. A. Al-Jumaili and K. K. Ahmed, "A review of antibiotic misuse and bacterial resistance in Iraq," *East. Mediterr. Health J.*, vol. 30, no. 10, pp. 1–12, 2024.
- [15] R. A. Moghnieh, D. Al-Tawil, C. Abdallah, and R. Kanj, "Carbapenem- and colistin-resistant Gram-negative bacterial pathogens in the Middle East: A review of the literature from 2010 to 2020," *Infect. Drug Resist.*, vol. 14, pp. 5023–5045, 2021. <https://doi.org/10.2147/IDR.S334521>
- [16] H. H. Jubair, Z. J. Hadi, and A. M. Almohana, "First report of colistin resistance gene *mcr-1* in carbapenem-resistant clinical isolates of *Klebsiella pneumoniae* in Iraq," *Medico-Leg. Update*, vol. 20, no. 2, pp. 461–466, 2020. <https://doi.org/10.37590/medlegupdate.v20i2.1148>
- [17] I. M. S. Al-Kadmy, B. M. Taha, and N. H. Hussein, "Prevalence of genes involved in colistin resistance in *Acinetobacter baumannii*: First report from Iraq," *Microb. Drug Resist.*, vol. 26, no. 6, pp. 616–622, 2020. <https://doi.org/10.1089/mdr.2019.0267>
- [18] Anonymous, "Current and emerging polymyxin resistance diagnostics: A systematic review of established and novel detection methods," *J. Appl. Microbiol.*, vol. 131, no. 5, pp. 2115–2130, 2021. <https://doi.org/10.1111/jam.15184>
- [19] R. T. Hamid and S. A. D. Ahmed Shanter Al-Qaysi, "Detection of colistin resistance and prevalence of *Mcr-1* to *Mcr-5* gene among clinical Enterobacteriaceae isolates in Iraq," *Biochem. Cell. Arch.*, vol. 21, no. 2, pp. 929–937, 2021.
- [20] M. Dadashi, Z. Dadashi, and H. Goudarzi, "Worldwide prevalence of *mcr*-mediated colistin resistance in *Escherichia coli*: A systematic review and meta-analysis," *Front. Microbiol.*, vol. 13, p. 923456, 2022. <https://doi.org/10.3389/fmicb.2022.923456>