

## Review Article

# PREVALENCE AND MOLECULAR CHARACTERIZATION OF COLISTIN RESISTANCE IN GRAM-NEGATIVE BACTERIA FROM NIGERIA HOSPITALS: A SYSTEMATIC REVIEW

## Abstract

Antibiotic resistance over the years, has emerged as a significant global public health problem, driven by the speedy adaptation of microorganisms to commonly prescribed antibiotics. Colistin, previously regarded as a last-resort antibiotic for treating infections caused by Gram-negative bacteria, is increasingly becoming resistant due to chromosomal alterations and the acquisition of resistance genes carried by plasmids, particularly the *mcr* genes. The mobile colistin resistance gene (*mcr-1*) was first discovered in *E. coli* from China in 2016. Since that time, studies have revealed that different variants of *mcr* genes ranging from *mcr-1* to *mcr-10*, mainly in Enterobacteriaceae from various parts of the world, which is a focal concern for public health. The co-presence of colistin-resistant genes with other antibiotic resistance determinants further conspire to frustrate treatment strategies and underscores the urgent need for enhanced surveillance and antimicrobial stewardship efforts. Therefore, a good grasp of the mechanisms driving colistin resistance and monitoring its global prevalence are essential steps in addressing the growing threat of antimicrobial resistance and preserving the efficacy of existing antibiotics. This review emphasizes the critical role of colistin as a last-choice antibiotic, elucidates the mechanisms of colistin resistance and the dissemination of resistant genes. It also explores the global prevalence of *mcr* genes, and evaluates the current detection methods for colistin-resistant bacteria. The objective is to shed light on these key aspects with strategies for combating the growing threat of resistance to antibiotics.

**Key words: Molecular Characterization, Antibiotic Resistance, Public Health, Gram-Negative Bacteria**

## I. INTRODUCTION

### 1.0 Background

“Antibiotics represent one of the most successful forms of therapy in medicine. But the efficiency of antibiotics is compromised by the growing number of antibiotic-resistant pathogens” (Roberts et al., 2015). “Deaths from **antibiotic resistance** is far outstripping even those of epidemics such as Ebola and COVID-19” (David, 2015). “COVID-19 is like a tip of an iceberg on the ocean, and the consequences of overuse of antibiotics are like the body of a mountain under water whose greatness has not yet been determined for humanity” (Mohammed et al., 2015). “The scientific community, mass media and political initiatives are creating awareness about antibiotic resistance. Despite these efforts and awareness antibiotic resistance is continuously increasing throughout the world” (Exner et al., 2015). “Multidrug resistance (MDR) has become a major problem for the treatment of bacterial infections and is becoming the greatest challenge to public health worldwide. MDR bacteria cause around 700 000 deaths worldwide every year and it is estimated they will cause 10 million deaths by 2050,

with a severe loss of economic resources” (Hartmann et al., 2015). “As per the World Health Organization (WHO) fact sheet, infections by antimicrobial-resistant organisms can result in failure of treatment, increased cost of medical treatment, increased hospitalization stay and increased socioeconomic burden” (Gandra et al., 2014). “Infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) have been reported to have increased significantly worldwide in recent years. The increase in MDR-GNB cases has become a serious challenge for health care professionals. Excessive use of antibiotics including the use without treatment indication is believed to be one of the major factors accelerating the spread of antibiotic resistance. Although Nigeria does not have comprehensive data on antimicrobial resistance, a situation analysis conducted by the Nigeria Centre for Disease Control (NCDC) in 2016 revealed that multidrug-resistant organisms were discovered from common healthcare-associated infections” (Hartmann et al., 2015).

“The National Action Plan for Antimicrobial Resistance, 2017-2022, found that bad use of antibiotics was common with 42% of adults and 46.7%–71.1% of five years’ children were given antibiotics without prescription, and 68.3% of adults used antibiotics following the prescription. Picture of AMR we are getting in Nigeria. Between 2019 and 2021, 5606 isolates were reported to have undergone antimicrobial susceptibility testing through the national human health sentinel surveillance system. Over these years, Carbapenem resistant enterobacteriaceae rate were 20-30% while ESBL rates were 60-80%” (Hamzat, 2021).

Global antibiotic consumption rates surged by 46%, indicating that the defined daily dose (DDD) per 1000 population per day rose from 9.8 to 14.3 between 2000 and 2018. In low- and middle-income countries (LMICs), antibiotic usage increased by 76% and is projected to continue rising by 2030 (Kpokiri, 2019).

The successes recorded in various aspects of modern medicine including surgeries, cancer chemotherapy and organ transplantation is being threatened by the emergence and spread of antibiotic resistant bacteria (Olowo et al., 2020). These antibiotic resistant bacteria constitute a serious threat to the public health in developed countries as well as in resource limited setting in developing countries, especially Nigeria (Busayo et al., 2020). Its prevalence is dangerously escalating worldwide leading healthcare practice towards a perilous era of post-antibiotics (Kadri et al., 2018).

“Although the emergence of antibiotic resistance is a natural phenomenon in most bacterial species, their spread is however being largely driven by negligent antibiotic use characterised by overuse and misuse in the healthcare systems, environment, and in agriculture/livestock practices. These factors alongside lack/inadequacy of infection control practices, overpopulation, poor sanitation and hygiene particularly in developing countries have exacerbated the problem” (Yusuf et al., 2014). “The poor regulatory system which enables easy accessibility to all antimicrobial agents over the counter in most developing countries including Africa and the distribution of sub-standard antimicrobials are some of the other factors driving the spread of drug resistant bacteria in the developing countries” (Yakubu et al., 2020).

Studies conducted in many African countries have revealed a growing prevalence of Gram-negative bacteria resistant to commonly prescribed antibiotics (Olowo et al., 2019).

Despite the untenable rate of antibiotic resistant bacterial infections reported in most countries, there is substantial gap in the surveillance of these infections in several Nigerian cities especially where limited research has been done on the prevalence of difficult to treat infections (Ahmed

et al., 2020). In the few studies conducted, a limited number of antimicrobial classes have been tested. This study therefore aimed to comprehensively investigate the prevalence of colistin resistance in Gram-negative bacterial infections among patients attending major hospitals in Nigeria(Larbi et al., 2020).

### **1.1 Colistin: A last resort Antibiotic:**

**Colistin** is one of the last remaining antibiotics that is still effective in killing bacteria and fighting infections such as pneumonia. It is deemed critically important for human medicine by the World Health Organization.

We surmise that mothers may have picked up these colistin resistant bacteria from the environment. We cannot speculate on the specific mechanism. The babies, meanwhile, could have picked up the bacteria from the hospital, the community, or from their mothers(Edward et al., 2021). It's not yet known if these colistin-resistant bacteria stay in the mothers or babies – but if they do this may increase their chances of acquiring future drug-resistant infections.

Kirsty, Edward and Timothy et al. collected samples from newborn babies and their mothers for a study between 2015 and 2017 from three hospitals in Kano and Abuja. The research is the largest ever screening of intestinal microbiota for colistin resistance in Nigeria.

Of the 4,907 samples analysed in their Cardiff and Oxford laboratories, they found that 1% of samples had genes conferring colistin resistance, across 41 mothers and eight babies. Although this is a low percentage, it is extremely worrying that any babies were carrying colistin-resistant bacteria within their first week of life.

### **1.2 Rationale for studying Colistin in Nigeria:**

Colistin is rarely used in hospitals and clinics in Nigeria. Therefore, research findings suggest that resistance may have emerged from the increasing use of colistin in agricultural settings in the country. Researchers are continuing their research with collaborators in Nigeria to further understand the levels of resistance in both the healthcare system and more broadly, hence the rationale to study within the hospitals in Southern Nigeria.

### **1.3 Objectives of study;**

To ascertain the prevalence of colistin resistance in Gram-Negative bacteria in the Nigeria Hospitals.

To assess the molecular characterization of colistin resistance in Gram-Negative bacteria in the Nigeria Hospitals.

To understand the mechanisms driving colistin resistance.

To estimate the growing threat of antimicrobial resistance and preserving the efficacy of existing antibiotics in Nigeria Hospitals.

To establish baseline data.

### **1.4 Significance of study;**

- This study will help suggest ideal use of colistin in treatment of bacteria infections

- This study will help identify the growing threat of antimicrobial resistance and preserving the efficacy of existing antibiotics in Nigeria Hospitals.
- This study will identify mechanisms driving colistin resistance..
- This study will serve as a baseline data for similar research within the area of study.

### **1.5 Scope of study;**

This study is designed to cover studies carried out within Nigeria. It will span from 2014 to 2024.

### **1.6 Inclusion criteria:**

Studies published in English.

Studies conducted in the past 10 years (2014 - 2024).

Studies focusing on patients with bacterial infections.

### **1.7 Exclusion criteria:**

Studies focusing on viral infection cases.

Studies conducted in pediatric populations.

Studies not reporting healthcare expenditure of quality of life outcomes.

### **1.8 Methodology:**

Systematic literature review

Electronic data bases(e.g., PubMed, Google scholar, Web of Science)

Hand searching of relevant journal

## **II. EPIDEMIOLOGY OF COLISTIN RESISTANCE**

### **2.1 Global Prevalence of Colistin Resistance:**

Bostanghadiri et al conducted a meta-analysis showed that there is a decrease in colistin resistance prevalence amongst isolates of *Acinetobacter baumannii*, the organism implicated for infections globally for the past two decades, though some countries have witnessed an increase in isolates that are colistin resistant. The study also revealed a regional trend in relation to the susceptibility of nosocomial infections caused by *Acinetobacter baumannii*- noting the increased resistance in regions where there is a heavy use of polymyxins like Qatar, Israel and France. This is a very disturbing fact as colistin is among the last line antibiotic therapy for extensively drug-resistant strains of *Acinetobacter baumannii* (Bostanghadiri et al., 2024). Folorunso et al conducted another study that showed that *Klebsiella pneumoniae* blood stream infection that is multidrug resistant was 70%. It was also shown that there was a moderate resistance to amikacin. It showed that

there is increased dependence on this carbapenem is due to increased resistance of this isolates to non-carbapenem antibiotics. (Folorunso et al., 2022) Additional review studies conducted in five continents namely Africa, Oceania, Asia, Europe, and America showed a prevalence rate of 2.27%, 0.32%, 25.49%, 66.72% and 5.19% respectively for mobile colistin resistance harbouring colistin resistant *E. Coli* (Dadashi et al., 2022) Another study also reviewed about 61 studies conducting a systemic review and meta-analysis. This showed a colistin-resistant rate for clinical

*S. maltophilia* isolates globally in Asian countries, Europe, north and south America to be 45%, 45% and 33% respectively. (Shams-abadi et al., 2023) It has also been shown that there is an increase in antibiotics resistance especially among the members of the Enterobacteriaceae family and nosocomial *P. aeruginosa* (Taleb et al., 2023). A study conducted by Narimisa et al showed a 1% global resistance of all isolates of *P. aeruginosa* to colistin. Patients with cystic fibrosis showed a 7% resistance to colistin which is the highest among the diseases examined. It also observed an increase in global colistin resistance from 2% to 5%. (Narimisa et al., 2024)

## **2.2 Colistin Resistance in Africa: A Focus on Nigeria**

One critical factor contributing to the rise of colistin resistance in Africa is the unregulated use of colistin in livestock. While colistin use in humans is relatively uncommon, it is frequently used in veterinary medicine without professional oversight. This inappropriate use creates selective pressure on bacterial populations, facilitating the acquisition and dissemination of resistance genes (Anyanwu et al., 2021). Also, many individuals resort to self-medication and patronize unqualified practitioners, which exacerbates the issue. The issue is compounded by the fact that counterfeit or low-quality antimicrobials are commonly available in the market, further contributing to the problem. This lack of oversight has resulted in the co-occurrence of *mcr* genes with resistance to other critical antibiotics, leading to multi-drug resistant (MDR) and pan-drug resistant (PDR) organisms, severely limiting therapeutic options.

A study on the molecular mechanisms of colistin resistance in Africa indicated that plasmid-mediated colistin resistance, which can be transferred between bacteria, is quickly becoming more prevalent in Africa. Research indicates that all ten *mcr* gene types (*mcr*-1 to *mcr*-10) have been detected within various isolates across the continent with *mcr*-1 being the most common genetic variant found in humans, animals, and environmental samples (Anyanwu et al., 2021). Studies have shown that banning non-therapeutic colistin use in developed countries has successfully reduced the spread of mobile colistin resistance (MCR) in humans and animals (Anyanwu et al., 2021). No such ban or regulation on colistin use exists in Nigeria where its use is currently widespread leading to a rise in the number of colistin-resistant strains (Ngbede et al., 2021). The unregulated use of colistin has been shown to encourage resistance in both pathogenic and commensal bacteria. The mobile colistin resistance (MCR) gene, a transferable, plasmid-mediated determinant, facilitates rapid horizontal transfer across species and genera, promoting widespread resistance (Anyanwu et al., 2021).

A study conducted by Agbo et al. (2024) in Nigeria highlights the rising prevalence of colistin resistance among bacteria in hospital wastewater, particularly in *Klebsiella* spp., *E. coli*, and *P. aeruginosa*. Specifically, colistin resistance was observed in 75% of *Klebsiella* spp., 83% of *E. coli*, and 100% of *P. aeruginosa* isolates. The study also stresses the importance of effective management of wastewater is vital to prevent the spread of resistance genes into the wider environment. To address this issue, it is important to enforce rigorous wastewater treatment procedures, consistently monitor wastewater for antibiotic-resistant bacteria, and create policies designed to limit the transmission of resistance genes from hospital effluents to the surrounding community.

Abattoir operations in Nigeria are generally unregulated by the relevant Government ministry/agency, thus limiting the management and operations of abattoirs to the patrons/proprietors, who have little or no training/knowledge of infection control practices (Omoruyi et al., 2023)

The result of a study done by Omoruyi et al, (2023) in Benin City, Edo State, Nigeria shows that plasmid-borne colistin-resistant and multidrug-resistant bacteria are prevalent in abattoir environments. This is an indication that abattoir facilities could be a source of human exposure to colistin-resistant bacteria, and efforts must be made to reduce the high dependence on antibiotics in farm animals.

Ngbede et al, (2021) in their study highlight "polyclonality," meaning that multiple unrelated bacterial lineages are present in the population. This diversity poses significant challenges for public health, especially in terms of tracking outbreaks and identifying sources of infection. When multiple unrelated strains are present, it becomes more complicated to determine where an outbreak originated and how it spread, which can hinder efforts to control and manage antibiotic resistance.

A review by Udeani and Ugah, (2021) found an overall high prevalence of colistin-resistant Enterobacteriaceae to be 45.8%, a high prevalence of colistin and tigecycline to be 22.2%, and 31.7% colistin and netilmicin resistant isolates. Among the risk factors that contribute to colistin, non-completion of antibiotics was found to be more significant ( $p = 0.039$ ).

### **2.3 Hospital acquired versus community acquired colistin resistance;**

The spread of bacterial infections vary significantly between community and hospital environments. Monitoring of bacterial pathogens and their resistance to antibiotics are important for effective control and management of infections. A study carried out in capetown revealed that the susceptibility of healthcare acquired pseudomonas aeruginosa and Acinetobacter baumannii complex isolates was <80% to all antibiotics apart from colistin (McKay & Bamford, 2015). Globally, the hospital environment faces a common challenge of HAIs (Hospital Acquired Infections) 87% Of HAIs are caused by gram negative organisms (Sydnor & Perl, 2011), Klebsiella spp., S aureus, Acinetobacter spp., and E. coli are the most implicated bacterial causing Hospital acquired infections in Africa(Irek et al., 2018). Concerns over toxicity led to a decline in polymyxin usage in the 1970s as safer and effective alternatives were available, but the increased rate of multidrug resistant infections and lack of novel antibiotics has prompted the reconsideration of clinical use of polymyxin B and polymyxin E (colistin) which are considered the last resort (Bergen et al., 2012).

The use of colistin in the prevention, control and treatment of infection in animals may be associated in the emergence of its resistance (Wangchinda et al., 2018) appropriate antimicrobial stewardship should be prioritized by veterinarians, especially with colistin as frequent exposure can pose a significant factor for emergence and spread of the resistant mcr1 – 10 genes which can also spread through contaminated water sources serving as reservoirs for infections (Sharma et al., 2022).

### **III. MECHANISM OF COLISTIN RESISTANCE**

#### **3.1 Chromosomal Mutations**

A mutation is a change or an alteration in the nucleic acid sequence of the genome of an organism or extrachromosomal DNA. Mutation is the first step of evolution which causes changes as small as the substitution of a single DNA building block, or nucleotide base, with another nucleotide base. Meanwhile, larger mutations can affect many genes on a chromosome. Alongside substitutions, mutations can also be caused by insertions, deletions, or duplications of DNA sequences. Colistin resistance poses a serious threat to public health, particularly in the context of antibiotic resistance. Studies have identified various mechanisms of colistin resistance, including plasmid-mediated mechanisms such as the MCR-1 gene (Liu et al., 2016). In a study carried out to evaluate Colistin resistance in *E. coli* strains from healthy animals in Korea, it was observed that the percentage of colistin resistance in *E. coli* isolates from livestock during 2005 and 2015 in Korea is mainly due to chromosomal mutations associated with LPS modification or unknown mechanisms occurring in sporadic clones, but not to the horizontal transfer of *mcr* genes or the spread of specific colistin-resistant clones. (Kim et al., 2019) The development of colistin resistance in different regions results from the high administration of colistin in clinical and veterinary settings. Colistin resistance in *E. coli* occurs through multiple chromosomal mutations and plasmid-mediated mechanisms. (Abavisani et al., 2023) In the study of *mcr*-negative *S. enterica* serovar Enteritidis strains, chromosomal mutations potentially involved in colistin resistance were identified by a genomic approach. Several chromosomal mutations were identified in the colistin-resistant *mcr*-negative *S. Enteritidis* strains in proteins involved in lipopolysaccharide and outer membrane synthesis and modification (RfbN, LolB, ZraR) and in a component of a multidrug efflux pump (MdsC). The plasmid-mediated mechanism and the potentially chromosomal mutations responsible for COL resistance were examined by whole-genome sequencing (WGS) in some isolates of this collection. (Fortini et al., 2022) In this study, none of the patients had a history of colistin treatment. This might indicate that the increasing use of colistin or polymyxin B in agriculture, livestock, and aquaculture has increased polymyxin resistance acquired via diverse mechanisms, i.e., not only by a horizontal transfer of *mcr*-positive plasmids but also by chromosomal mutations that confer high-MIC resistance in the absence of *mcr*. (Poirel et al., 2017). Colistin resistance of the clinical isolates was not only associated with *mcr-1* but also with chromosomal mutations, although the patient histories confirmed that they had not previously received polymyxin treatment (Nuhu et al., 2024). Resistance mechanisms are presumed to be linked to chromosomal mutation untransferable via horizontal gene transfer. (Poirel et al., 2017) Although *mcr* is a plasmid-mediated gene, recently Zurfluh et al identified the *mcr1* gene on chromosomes of *E. coli* strains. Therefore, there is a hypothesis that this gene can be integrated in the genome of some isolates (Zurfluh et al., 2016).

#### **3.2 Plasmid-Mediated Resistance (*mcr-1*, *mcr-2*, etc.)**

Polymyxins are cyclic cationic lipopeptides with bactericidal activity with colistin being the most widely used (Materon et al., 2023). The widespread emergence of colistin resistance in bacteria has garnered global attention, as colistin is a critical last-resort antibiotic for infections caused by resistant pathogens. Mobile colistin resistance (*mcr*) is the main mechanism behind colistin

resistance and has spread globally, drawing significant attention (Liu et al., 2024). Mcr stands for “plasmid-mediated colistin resistance,” describing the gene’s ability to avoid the effects of colistin and share this ability with other bacteria and several plasmid-mediated colistin resistance genes (mcr-1, 2, 3, 4, 5, 6, etc) have been identified across human and animal species and the descriptions -1, -2, -3, and -4 indicate different DNA sequences.

The discovery of plasmid-mediated mobile colistin resistance (mcr) genes has intensified public health concerns worldwide, as these genes can spread through horizontal transfer, increasing the risk of global dissemination (Hussein et al., 2021). Since the initial discovery of a mobilized colistin resistance gene (mcr-1), several other variants have been reported, some of which might have circulated beforehand (Martiny et al., 2022). Currently, ten slightly different variants of the mcr-1 gene (mcr-1 to mcr-10) have been identified in different bacteria isolated from animals, foods, farms, humans, and the environment (Hussein et al., 2021). They are found across over 60 countries in 6 of the 7 continents except Antarctic (Anyanwu et al., 2021).

The mcr-1 was the first plasmid-mediated colistin resistance gene to be identified, and it was initially identified on an IncI2 plasmid in *Escherichia coli* and *Klebsiella pneumoniae* in China. Its widespread presence now indicates that colistin use has likely accelerated the spread of mcr-1 among both animals and humans (Yang et al., 2018). Currently, more than 25 different variants of the mcr-1 gene have been reported, differing from mcr-1 by one or two amino acid changes (Mondal et al, 2024).

Nine more mcr genes and their variants have been identified across various Enterobacterales species. These genes encode PEA transferase enzymes with amino acid identities ranging from 32% to 88%, with MCR-1 being the most prevalent in clinical isolates (Materon et al., 2023). In a review study by Mondal et al, (2024), it was observed that the mcr-2 gene containing 1617 base pairs, and a phosphoethanolamine transferase activity was present in IncX4 plasmid and was, for the first time reported to be highly prevalent in bovine and porcine colistin-resistant *E. coli*, collected from Belgium. Evidence from phylogenetic analysis/studies revealed that this gene was distinct from mcr-1 and shared 76.74% similarity with it. It was co-harbored with a lipid phosphatase gene, which shared a strong homology with the phosphatase gene present in *Moraxella* spp.

The mcr-3 gene, spanning 1626 base pairs, was initially identified in *E. coli* isolated from pigs, exhibiting 47% and 45% nucleotide similarities with the mcr-2 and mcr-1 genes, respectively (Mondal et al, 2024). The mcr-2 and mcr-3 genes were identified on conjugative plasmids in Enterobacteriaceae, meaning they can be easily transferred between bacteria, thus spreading resistance.

The mcr-4 gene, spanning 1626 base pairs, was initially identified in *S. enterica*, particularly in a monophasic serovar Typhimurium isolate from a pig at slaughter in Italy, and in *E. coli* strains from Spanish and Belgian piglets, indicating its significant dissemination across Europe (Materon et al., 2023). The mcr-3 has high amino acid identity with phosphoethanolamine transferases in other Enterobacteriaceae and *Aeromonas* species. The plasmid containing mcr-3

was transferred to another *E. coli* strain via conjugation, highlighting its potential for spread. Additionally, a truncated transposon, TnAs2, was found near *mcr-3* in pWJ1 and observed in various bacterial isolates across countries, suggesting that *mcr-3* could disseminate widely in Enterobacteriaceae and possibly in *Aeromonas*, which may serve as a reservoir (Yin et al., 2017).

In a study by Hammerl et al., (2018), two newer variants, *mcr-4* and *mcr-5* were identified in different strains of *Salmonella enterica*. These genes were located on non-conjugative ColE plasmids, which are not capable of self-transfer but can still spread resistance under certain conditions. *Mcr-4* can be transmitted with the help of a “helper” plasmid, *mcr-5* can be mobilized by a Tn3-type transposon, a mobile genetic element that can help the gene integrate into other plasmids or chromosomes. This transposon, Tn6452, was found on the chromosome and plasmids of a strain known as *Salmonella Paratyphi B* (dTa+).

The *mcr-6* Gene *mcr-6* gene contains 1617 base pairs and exhibits phosphoethanolamine-lipid A transferase activity. The genetic variant of the *mcr-2* gene present in *M. pluranimalium*, pigs’ isolate identified which was later named *mcr-6.1* in Great Britain. To date, only one variant of this gene, *mcr-6.1*, has been reported. Remarkably, *mcr-6* is the only variant of the mobile colistin-resistance gene that has not been reported from China yet (Mondal et al, 2024).

The *mcr-7* was discovered in *Klebsiella pneumoniae* strains from chickens in China. This gene is located on an IncI2-type conjugative plasmid, facilitating its transfer between bacteria (Yang et al., 2018). It contains 1620 base pairs and the e protein product of this gene shares the highest amino acid sequence similarity with *mcr-3*, reaching 70% among all other *mcr* variants (Mondal et al, 2024).

The *mcr-8* gene comprises of 1698 base pairs and located on a conjugative plasmid, was first identified in a *K. pneumoniae* strain resistant to both colistin and carbapenem that was obtained from samples of human sputum and pig feces in China. In another study, the *mcr-8* gene variant (*mcr-8.3*) was found to co-occur with *mcr-3* gene variants (*mcr-3.21*, *mcr-3.26*, and *mcr-3.28*) in strains of *K. pneumoniae* identified from healthy human stool samples collected in Thailand and Laos (Mondal et al, 2024).

The *mcr-9* gene This gene, characterized only recently in 2019, was found in an *S. enterica* strain of serotype Typhimurium that was first isolated from a patient in Washington State in 2010. When the predicted protein structures of MCR-9 and other MCR homologues were compared, it was found to share significant similarity with *mcr-3* (Mondal et al, 2024). Although the *Salmonella* strain containing *mcr-9* did not show resistance to colistin at a standard testing concentration (2 mg/L), scientists wanted to see if *mcr-9* could still confer resistance in other settings. To test this, they introduced *mcr-9* into a colistin-sensitive *Escherichia coli* strain (NEB5 $\alpha$ ) and activated it using IPTG, a chemical that turns on gene expression. When *mcr-9* was expressed in this *E. coli* strain, it conferred some level of resistance to colistin at concentrations of 1, 2, and 2.5 mg/L, although the resistance was weaker than that seen with *mcr-3* (Carroll et al., 2019). Further analysis compared the protein structures of *mcr-9* with other *mcr* variants (*Mcr-1* through *Mcr-*

9) and found that *mcr-9*, *mcr-3*, *mcr-4*, and *mcr-7* shared considerable structural similarities. This suggests that *mcr-9* has the potential to cause colistin resistance in Enterobacteriaceae bacteria and should be included in monitoring efforts for plasmid-mediated colistin resistance, as it may contribute to the spread of antibiotic resistance in these bacteria.

Mondal et al, (2024) reported in their review that the *mcr-10*-harboring isolates were identified during a comprehensive screening of 941,449 genomes of bacteria from the GenBank database, primarily among clinically threatening *K. pneumoniae* ST11 strains. This discovery underscores the sporadic distribution of *mcr-10* across various sources worldwide, with a notable prevalence in human-related contexts, raising serious clinical concerns.

### **3.3 Efflux Pumps and Other Resistance**

Mechanisms Efflux pumps are transport proteins, which are active pump systems responsible for removing toxic substances from cells to extracellular environment (Webber M.A & Piddock L.J.V., 2003). Overexpression in these pumps are recognised to be linked to a resistance against drugs (Sun, Jand Deng & Zand Yan, A., 2014). Efflux pumps reduces the drug concentration without modification of the antibiotic (Fraqueza M.J., 2015). The efflux pump mechanisms are caused by mutations in the regulatory genes (Zarakolu P., 2003; Blanco P. et al., 2016). Bacterial efflux pumps are classified into five families: the resistance-nodulation-division (RND), the major facilitator superfamily (MFS), the ATP (adenosine triphosphate)-binding cassette (ABC), the small multidrug resistance (SMR), and the multidrug and toxic compound extrusion (MATE) family (Poole K., 2007; Piddock L.J., 2006; Putman M et al., 2000). The RND is found in Gram-negative bacteria, while the others are found in both Gram-positive and negative bacteria (Handzlik J., 2013). The colistin resistance mechanisms in gram-negative bacteria have been summarized in many previous reviews (Jeannot et al., 2017; Gogry et al., 2021). In general, the colistin resistance mechanism mainly includes two steps. The first step - point mutations in the TCS *PmrAB*, *PhoPQ*, *CrrAB*, and other lipid A modification coding genes located in chromosome loci produce more positively charged phosphoethanolamine to be added to the outer membrane lipids, resulting in weakened binding to colistin. Mutational inactivation and truncation of the insertion sequence of the *PhoQ* kinase inhibitor *mgrB* may also be effectively involved in this process and the second step - phosphoethanolamine transferase encoded by *mcr* can also mediate the modification of the outer membrane (lipid A) (Liu et al., 2016). A study on *A. hydrophila* suggests that the outer membrane lipoprotein-encoding gene, *MlaA*, may be associated with high levels of colistin resistance (Liu et al., 2021). Also, Zeng et al. found that the *RpoE* stress system mediated colistin resistance in *E. coli* without disturbing the lipid A profile (Zeng et al., 2023). The diversity of colistin resistance mechanisms was discovered by colistin-degrading proteases (Lee et al., 2022). Colistin, as a cationic polypeptide compound, acts mainly on the outer membrane of bacteria carrying a negative charge (Falagas and Kasiakou, 2005). However, the lipid modification mediated by mutation and the inactivation of the two-component regulatory systems (TCS: *PmrAB* and *PhoPQ*) and *mgrB* are common causes of reduced susceptibility to colistin in bacteria. Plasmid-mediated horizontal transfer of *mcr* and its variants in bacteria promotes the rapid emergence of colistin resistance. Previous studies have shown that efflux pump inhibitors (EPIs) CCCP and NMPs reverse colistin resistance, suggesting the role of efflux pumps in colistin resistance (Naha et al., 2022). Efflux pumps, such as the

KpnEF, AcrAB and Sap proteins, have been reported in Enterobacteriaceae. By activation of these pumps, resistance to colistin is increased (Chambers J.R. & Sauer K., 2013; Srinivasan V.B. & Rajamohan G., 2013). The efflux pump KpnEF is a member of the Cpx regulation which is responsible for capsule synthesis in *K. pneumoniae* and belongs to the SMR protein family (Baron S. et al., 2016). In *K. pneumoniae*, this pump is mediated by colistin resistance and other antibiotics, including ceftriaxone, erythromycin, and rifampicin (Srinivasan V.B. & Rajamohan G., 2013).

#### **IV. MOLECULAR CHARACTERIZATION OF COLISTIN-RESISTANT GRAM-NEGATIVE BACTERIA**

##### **4.1 PCR-Based Detection of Resistance Genes;**

The most sensitive for determining antibiotic resistance by assessing the presence of resistance genes or mutations conferring resistance are the molecular biology methods. The main mutations for Enterobacteriaceae species are located on genes coding the two-component systems PmrA/PmrB and PhoP/PhoQ. Specifically, mutations in the mgrB gene with the presence of insertional sequences, appeared to be the main resistance mechanism observed in *K. pneumoniae* strains. Screening of potential mutations on these chromosomal genes is done by amplification and sequencing which takes 3 days requiring all genes to be tested. Sequenced amplicons are then compared by the BLAST tool against the NCBI database to screen possible mutations compared to wild-type genes (Bardet L. and Rolain J., 2018). The discovery of the acquired gene mcr-1 justifies the use of molecular detection with RT-PCR, a rapid quantitative technique to detect the presence of the gene (Skov R.L. et al., 2016). Scientists have used primers of the original study (Liu Y.Y. et al., 2016), or designed their own primers for standard PCR (Rojas L.J et al., 2016; Bontron S. et al., 2016; Chabou S. et al., 2016; Quan J. et al., 2017; Falgenhauer L. et al., 2016; Ye, H. et al., 2016;), or RTPCR, based on SYBR Green assays (Don`a V et al., 2017; Bontron S. et al., 2016; Lekunberri, I et al., 2017), TaqMan probe (von Wintersdor C.J.H et al., 2016; Payne M et al., 2016; Chabou S. et al., 2016; Irrgang, A. et al., 2016), or other FAM-labelled probe (Yang Y.Q. et al., 2017; Wong S.C.Y et al., 2016; Nijhuis, R. H. T et al., 2016; Yang, D et al., 2017) or HEX-labelled probe (Snesrud, E. et al., 2017). Xavier et al. designed primers to screen mcr-2 (Xavier B.B. et al., 2016). Some designed their own primers for standard PCR (Sun J. et al., 2017; Simmen, S. et al., 2016), and a study developed a TaqMan assay for qPCR (Roschanski, N. et al., 2017). These primers were designed for detecting mcr-3 (Yin W. et al., 2017), mcr-4 (Carattoli A. et al., 2017), and mcr-5 genes (Borowiak M. et al., 2017) by standard PCR. A recent study described a multiplex SYBR Green real-time PCR assay for the simultaneous detection of mcr-1, mcr-2, and mcr-3 genes (Li J. et al., 2017; Rita Rebelo, A. et al., 2018). PCR also detect plasmid carrying mcr genes (Sekizuka, T. et al., 2017). This sequence may form a composite transposon that can potentially move as one complete unit (Falgenhauer L. et al., 2016; Doumith, M et al., 2016; Stoesser, N et al., 2016). This insertion sequence appears to be a key component of the mobilome, and its presence is not systematic (Snesrud, E. et al., 2017). Li et al. identified the ability of the Tn6330 transposon to generate circular ISAp11-mcr-1-orf (Li R. et al., 2017). Specific primers were developed to screen the upstream presence of this IS transposon by PCR and Sanger sequencing (Veldman, K. et al., 2016; Campos, J. et al., 2016; Anjum, M.F. et al., 2016). Others have also designed their own system to directly screen on plasmid carrying mcr-gene type IncX4 (Yang Y.Q. et al., 2017; Poirel, L et al., 2016).

## 4.2 Whole-Genome Sequencing and Phylogenetic Analysis

The use of whole-genome sequencing and phylogenetic analysis has proven to be vital in the molecular characterization of colistin-resistant Gram-negative bacteria. These approaches have improved researchers ability to identify resistance mechanisms, track the spread of resistant strains, and enhanced the understanding of the genetic factors contributing to antimicrobial resistance. Study by (Hahm et al., 2022) have emphasized the increasing prevalence of colistin-resistant Gram-negative bacteria, particularly in *Acinetobacter baumannii* and *Escherichia coli*. Whole-genome sequencing (WGS) has emerged as a powerful tool for describing resistance mechanisms and analyzing molecular epidemiology. According to (Kim et al., 2019) Phylogenetic analysis based on whole-genome sequencing and multilocus sequence typing revealed the similarity of the isolate to strains from different parts of the world, emphasizing the global nature of antimicrobial resistance dissemination. This method is based on the detection of specific markers associated with colistin resistance, providing a quick and precise method for identifying resistant strains. (S et al., 2021) According to (Shelenkov A et al., 2020) molecular typing and whole-genome sequencing were used to analyze clinical *Klebsiella pneumoniae* isolates in the Russian Federation. The analysis identified new multilocus sequence typing-based sequence types, multidrug-resistant isolates, and colistin-resistant isolates, underscoring the importance of genomic analysis in understanding the epidemiology of resistant bacteria. The development of colistin resistance in carbapenem-resistant isolates is particularly concerning, as it further narrows treatment options and necessitates improved antimicrobial stewardship and infection control measures (Fam et al., 2020). WGS analysis has revealed the prevalence of high-risk lineages, such as international clone 2 in *A. baumannii*, often harboring multiple resistance genes like blaOXA-23-like and blaNDM (Fam et al., 2020).

## 4.3 Bioinformatics Tools for Resistance Prediction;

The rise in antibiotic-resistant bacteria is becoming more widespread and presents a major threat to global public health. Without intervention, the annual death toll from these infections could climb to 10 million by 2050 (Yagimoto et al., 2024). Bioinformatics is a hybrid science that links biological data with techniques for information storage, distribution, and analysis to support multiple areas of scientific research, including biomedicine. It is fed by high-throughput data-generating experiments, including genomic sequence determinations and measurements of gene expression patterns (Lesk, 2024). Predicting the underlying resistance mechanisms of antibiotic resistance genes (ARGs) is crucial for understanding and combating this problem. Predicting resistance refers to identifying the likelihood that a microorganism or a cell (such as cancer cells) will resist the effects of a drug. It involves analyzing genetic, proteomic, or structural data to detect specific mutations, resistance genes, or changes in protein structures that are known to confer resistance. By identifying these markers, scientists and clinicians can anticipate which treatments might fail, thereby informing more effective treatment strategies and reducing the risk of ineffective or harmful treatments (Yagimoto et al., 2024).

Phenotypic antimicrobial susceptibility testing (AST) is the classic method to detect AMR, but in the last few years, Whole Genome Sequencing-based AST (WGS-AST) has emerged as a fast and accurate method for AMR detection (Seoane & Bou, 2021). With advances in whole genome sequencing (WGS), researchers can use sequence alignment methods—like best-hit

approaches—to detect AMR genes in both individual bacterial genomes and complex metagenomic datasets. These alignment methods work by comparing DNA sequences in samples to known AMR genes in public databases, effectively identifying highly conserved AMR genes with few false positives. However, these methods can struggle with AMR genes that differ significantly from known sequences, resulting in high false-negative rates in cases where genes are less conserved. WGS-AST techniques are not routinely performed in most clinical microbiology laboratories, because of their high cost, the need for skilled personnel, the poor quality of the data obtained, and the difficulty in interpreting those data (Seoane et al., 2021). Another major challenge with whole genome sequencing (WGS) is that it generates vast data, making it difficult to detect unknown antibiotic resistance (AMR) genes or variants. Luckily, tools like Resfinder, PARGT, AMRfinder, and the Comprehensive Antibiotic Resistance Database (CARD) allow users, even those without advanced bioinformatics skills, to analyze WGS data and identify AMR-related genetic elements like mutations or horizontal gene transfers (Seoane et al., 2021). These tools rely on curated AMR databases but differ in algorithms and data types. However, a study by Doyle et al. found that results can vary significantly across labs due to differences in software and analysis methods, leading to inconsistent results compared to traditional antimicrobial susceptibility testing (AST). These inconsistencies may also stem from data quality issues and variations in interpretation.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) suggests that WGS alone is currently unreliable for precise AST predictions and recommends using epidemiological cut-off values (ECOFF) rather than clinical breakpoints. Currently, WGS-based AST isn't standard practice in most clinical labs due to high costs, the need for skilled analysts, and challenges with data quality and interpretation. But as costs fall and bioinformatics tools advance, WGS could become a routine method for AMR detection, with better data analysis translating into clearer and more actionable insights.

To address some of the challenges of WGS, machine learning offers a powerful alternative, especially when applied to large and complex datasets from metagenomic studies. A machine learning algorithm can be trained to detect AMR genes using positive training data (protein sequences of known AMR genes) and negative training data (protein sequences of non-AMR genes). These models analyze diverse features—characteristics of the proteins, such as sequence motifs or structural properties—that distinguish AMR from non-AMR genes. An effective machine learning model aims to learn which features are most informative for differentiating AMR genes, allowing it to generalize to new data. In metagenomic contexts, this means that machine learning can potentially identify novel or less familiar AMR genes, even when they have low similarity to known sequences, reducing the likelihood of false negatives. This flexibility makes machine learning a robust tool for detecting AMR genes across diverse microbial communities, contributing to a more comprehensive understanding of AMR presence and prevalence in environments that are difficult to analyze with traditional methods alone.

PARGT is an open-source tool developed to predict antimicrobial resistance (AMR) genes in bacteria. It combines Python 3 and R, with R scripts used for analyzing protein features and creating machine-learning models, while Python coordinates these scripts, generates position-

specific scoring matrix (PSSM) features, and provides the graphical interface. PARGT emphasizes protein features according to their classification relevance and includes all necessary bioinformatics tools for feature extraction. Using its GTDWFE algorithm, PARGT selects the most informative features for AMR predictions. Users can add new AMR and non-AMR sequences to its training data, with the model automatically updating to potentially improve accuracy. To save processing time, PARGT uses the UniProt database, which includes 538,585 protein sequences, rather than a larger database, for PSSM and structural feature generation (Chowdhury et al., 2020).

ResFinder is a tool that detects acquired antibiotic resistance genes and chromosomal mutations responsible for antimicrobial resistance in bacterial DNA sequences, whether complete or partial. Since its launch in 2012, ResFinder has seen several updates, including code and database enhancements, the addition of point mutation detection for specific bacterial species, and phenotype predictions for select species. As of September 28, 2021, ResFinder has been used 820,803 times by users from 61,776 IP addresses across 171 countries, demonstrating its global relevance. The developers aim to keep ResFinder free and plan to expand its capabilities, such as offering phenotypic predictions for more bacterial species in the future (Florensa et al., 2024).

## **V. CLINICAL IMPLICATIONS AND TREATMENT OPTIONS**

### **5.1 Colistin Treatment Failure and Clinical Outcomes.**

Studies reveal high colistin treatment failure rates, ranging from 20% to 40%, depending on patient characteristics and infection severity (Falagas et al., 2014). Factors associated with failure include bacterial resistance mechanisms like *mcr-1*-mediated resistance, pharmacokinetic challenges in achieving effective plasma concentrations, monotherapy, and host factors such as compromised immunity. Furthermore, infections in critically ill patients with comorbidities tend to have poorer responses to colistin therapy (Barnes et al., 2019).

#### **Resistance**

One major problem is that many Gram-negative bacteria that were effectively handled by colistin now have genes that are resistant to colistin largely attributed to the improper and excessive use of antibiotics in both medical and agricultural settings. (Mondal et al., 2024). To combat colistin resistance, innovative solutions are essential.

#### **Colistin Side effects**

Colistimethate sodium (CMS) therapy, commonly used to treat multidrug-resistant gram-negative bacterial infections, is associated with significant adverse reactions, particularly nephrotoxicity and neurotoxicity. Nephrotoxicity, the most frequent adverse reaction, typically manifests within a median timeframe of 2.5 to 10 days after starting treatment. Fortunately, renal function often recovers within 3 to 9 weeks once treatment is discontinued. The mechanism underlying CMS-induced nephrotoxicity is linked to its antibacterial action. Colistin increases the permeability of epithelial cell membranes in the kidneys, leading to an influx of

ions and water. This influx causes cellular swelling, leakage of cell contents, and eventually cell death, a process thought to be dose-dependent. Due to the association between nephrotoxicity and increased mortality risk, monitoring renal function is standard practice during CMS therapy, emphasizing the need for careful dose management and frequent kidney function assessments to mitigate risks (Sadyrbaeva-Dolgova., et al 2022).

## **5.2 Alternative antibiotics and combination therapies;**

The most pressing concern in AMR lies with gram negative pathogens, of which colistin is used as last resort. However, combination therapies like caftazidime – avibactam, meropenem – nvarbobactam and ceftolozane –tazobactam provide effective solution against multi drug resistant strains of these pathogens particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae* (Lutgring et al., 2019).

The emergence of this colistin resistant strains evoked innovative solutions such as development of new molecules, combination of colistin with other drugs, exploring new indications for existing drugs, nanotechnology, phage –based approach (Cardozo et al., 2015), studies have identified Fluopsin C, a bioactive compound derived from *Streptomyces* and *Pseudomonas*, as a highly effective antimicrobial agent against Gram – positive, Gram – negative and multi drug resistant bacteria (Navarro et al., 2019).

Combination of colistin with other antibiotics are popularly called combinatorial therapy (Almutairi, 2022) tigecycline, meropenem, gentamycin or fosfomycin are other antibiotics that are mostly combined with colistin (Dizbay et al., 2019), combination of colistin and rifampicin or macrolides demonstrated effective treatment in *mcr-1* positive *Klebsiella pneumonia* in two different mouse models reflecting their potent synergism (MacNair et al., 2018) the *mcr* (mobilized colistin resistance) gene is the strain responsible for colistin resistance (Gharaibeh & Shatnawi, 2019) also, a combination of colistin and tigecycline exhibited substantial antimicrobial activity against *E.coli* harbouring *mcr -1* in contrast to when the antibiotics were used independently, even in higher doses (Zhou et al., 2020).

Research has shown that antibiotic monotherapy is often inadequate against drug resistant bacteria including *klebsiella pneumonia* thus also encouraging combination therapy as an alternative. Resistant isolates of *K. pneumonia* were tested against 16 conventional antibiotics alone and also in combination with colistin. Colistin combined with amikacin or fosfomycin showed synergy against 72.2%, combination with levofloxacin showed synergism against 90% of the isolates, combination with ciprofloxacin or tobramycin demonstrated only 45.45% synergism, combination with meropenem, ceftazidime, moxifloxacin or piperacillin showed 81.82% synergism. This establishes the fact that combination therapy regimen is effective and provides a promising strategy against multi drug resistant bacteria (Ontong et al., 2021). But as AMR is still on the increase, new strategies are being adopted for treating infections such as combination of polymyxin B with non antibiotics to improve its efficacy, three antidepressants (amitryptilline, imipramine and setraline) and four antipsychotics (chloropromazine, clonazepan, haloperidol and levopromazine) together with polymyxin B showed different levels of

antibacterial action against 20 gram-negative organisms which is not obtainable with polymyxin B alone. Synergistic antimicrobial actions were observed when polymyxin B was combined with setraline, chlorpromazine or levopromazine against multi drug-resistant *A.baumannii*, *E.coli* and *K. pneumoniae* (Otto et al., 2019). The best approach to combat AMR remains public awareness on the dangers of antibiotics abuse (Gharaibeh & Shatnawi, 2019).

### **5.3 Infection Control and Stewardship Programs;**

The increasing use of colistin in clinical settings, including the veterinary clinics, has led to the rise of colistin resistance. Studies have shown that the prevalence of colistin resistance has increased among Enterobacteriaceae. (Aghapour Z. et al., 2019). Widespread transmissible colistin resistance is a concern in the current era of MDR gram-negative infections because colistin is used to treat infections caused by these organisms, regardless of its nephrotoxicity and limitations in determining susceptibility and appropriate dosing regimens (Landersdorfer C.B. & Nation R.L., 2015). Colistin is used as the last alternative antimicrobial against MDR and PDR (pan-drug-resistant) Gram-negative infections (Dixon R.A. & Chopra I., 1986; Li J. et al., 2006; Antoniadou A. et al., 2007). Different bacterial species have developed resistance due to the inappropriate use of colistin. Prevention strategies play a crucial role in the management of colistin-resistant genes. These strategies include: genotyping and rapid diagnosis, surveillance culture, infection control measures and antimicrobial stewardship programme (Sharma J. et al., 2022). Antimicrobial stewardship is a proven way to curb antimicrobial resistance. Education of human and animal health workers and the public on the judicious use of antibiotics, conduct of antimicrobial sensitivity test before antibiotics prescription (Madubuike U. A. et al., 2021). Recommendations in response to the rise and spread of plasmid-mediated colistin resistance include preserving colistin use for definitive treatment based on susceptibility testing and use of PK/PD indicators to ensure appropriate Dosing (Al-Tawfiq J.A. et al., 2016). Clinicians should be vigilant to the likelihood of colistin resistance among MDR bacteria and the development of colistin resistance through mutation or adaptation mechanisms. (Aghapour Z. et al., 2019).

## **VI. CHALLENGES AND FUTURE DIRECTIONS**

### **6.1 Limitations of Current Detection Methods;**

Colistin-resistant Gram-negative bacteria pose a significant challenge in healthcare settings due to limited treatment options. Current methods of identification for colistin resistance differ in their efficacy and reliability, leading to difficulties in precisely identifying these resistant strains (Smith et al., 2021). Mechanisms of resistance in Gram-negative bacteria, such as decreased membrane permeability and genomic mutations, contribute to the challenge of detecting and characterizing colistin resistance (Molecular Methods for Detection of Antimicrobial Resistance, n.d.). Mechanisms of resistance in Gram-negative bacteria, such as reduced membrane permeability and genomic mutations, contribute to the challenge of detecting and characterizing colistin resistance (Husna et al., 2023). Phenotypic methods include agar-based media, MIC-determiners, and rapid colorimetric tests, while molecular methods encompass PCR, LAMP, and whole-genome sequencing (Osei Sekyere, 2019). For well-equipped laboratories, multiplex PCR or real-time PCR assays, combined with phenotypic methods, are suggested for comprehensive detection. Ongoing surveillance of carbapenem-resistant Gram-

negative bacteria is essential to combat this global epidemic and prevent the spread of antimicrobial resistance (Fam et al., 2020). Current research aims to develop more efficient, sensitive, and specific diagnostics to address the growing concern of colistin resistance (de Maio Carrillho et al., 2017). Additionally, the clinical efficacy of colistin in treating Gram-negative bacterial infections, such as those caused by *E. coli*, may be diminishing due to the spread of resistance mechanisms (Iroha et al., 2023). In the clinical setting, MALDI-TOF MS has emerged as a reliable tool for detecting colistin-resistant pathogens, particularly Gram-negative bacteria (Osei Sekyere, 2019). However, there is a lack of consensus on the methodology for colistin susceptibility testing, leading to challenges in accurately identifying resistant strains (Gogry et al., 2021). In Gram-negative bacteria, mechanisms of resistance may involve genomic mutations that decrease membrane permeability, and also the exchange of carbapenemase genes on plasmids (Husna et al., 2023). Moreover, the occurrence of carbapenem-resistant strains further complicates the treatment of infections caused by multidrug-resistant Gram-negative bacteria (Suwantararat & Carroll, 2016). Overall, the molecular characterization of colistin-resistant Gram-negative bacteria presents significant challenges in current detection methods. Enhancing the precision and efficiency of these methods is essential for guiding appropriate treatment plans and combating the spread of antibiotic resistance in clinical settings (Smith et al., 2021). Further research is needed to improve the detection and characterization of colistin-resistant Gram-negative bacteria to inform treatment strategies and mitigate the impact of antimicrobial resistance in clinical settings.

## **6.2 Need for Enhanced surveillance and reporting**

AMR surveillance aims to monitor change in bacterial populations, to enable early identification of resistant strains that are harmful to the public, thereby facilitating outbreak detection and intervention (Home • Antimicrobial Resistance (AMR), n.d.). Colistin susceptibility testing should ideally be a part of national strategies for AMR surveillance. Countries with advanced AMR surveillance programs and laboratories can improve in the effort to monitor colistin resistance in bacteria from food, animals and environmental samples, regularly. The technical challenge of detecting colistin resistance makes it unsuitable for screening in low-resource settings and developing countries. Laboratories should therefore store suspicious isolates and seek collaborations with reference labs or WHO centers for expert analysis to detect the possible presence of the *mcr* resistant strains. Healthcare facilities that experience carbapenem-resistant or multidrug-resistant infections, especially Enterobacteriaceae, *Acinetobacter baumannii* and *P. aeruginosa*, can conduct regular colistin resistance surveys in order to optimize and inform local antimicrobial policies (Global Antimicrobial Resistance Surveillance System (GLASS) The Detection and Reporting of Colistin Resistance, n.d.). The following pathogens should be screened for colistin resistance, except bacteria with intrinsic colistin resistance;

Carbapenem-resistant Enterobacteriaceae

Other gram-negative bacteria resistant to carbapenems and

When treatment with colistin must be considered (GLASS The Detection and Reporting of Colistin Resistance Second Edition Global Antimicrobial Resistance and Use Surveillance System (GLASS), n.d.). Surveillance among nations should be harmonized to a "One Health" approach to combat this threat to both humans, animals and the environment globally (Thakur & Gray, 2019) because the health of human beings is dependent on that of both animals and the

environment as acknowledged by the US CDC (Thompson & Kaplan, Encyclopedia of food and agricultural ethics).

### **6.3 Research Gaps and Priorities for Nigeria**

The use of colistin drug in human medicine and animal husbandry, has a serious impact on the rise and spread of colistin resistance among Gram-negative bacteria. (Gogry F.A., et al., 2021). Researchers and laboratory scientists should develop new molecules/antibiotics, with better effects and tolerance than colistin. The combination of meropenem and colistin has shown a synergistic effect against antibiotic-resistant Gram-negative bacteria and has the potential to reduce the development of resistance (Aghapour Z. et al., 2019; Lomonaco S. et al., 2018; Skov R.L., et al., 2016; Sharma D. & Fluopsin C., 2020). Collaboration between the various health and veterinary sector to prevent colistin resistance is of importance also (Andersson D. I. et al., 2014; Robinson T.P. et al., 2016; Larsson D.G.J. et al., 2014).

## **VII. CONCLUSION**

### **7.1 Summary;**

Colistin, previously regarded as a last-resort antibiotic for treating infections caused by Gram-negative bacteria, is increasingly becoming resistant among Southern Nigerian population due to chromosomal mutations and the acquisition of resistance genes carried by plasmids, particularly the *mcr* genes.

This antibiotic is not widely prescribed in Nigerian hospitals; however, it is primarily used in veterinary treatment and for preventing bacterial infections in livestock, which humans consume.

These results indicate that there may be insufficient infection control measures in Nigeria hospitals and a potential issue with the indiscriminate use of colistin in both humans and animals, which can complicate the treatment of bacterial infections.

Therefore, it is recommended to avoid the unnecessary use of this antibiotic to prevent the potential increase in colistin resistance in the future.

### **7.2 Implications for Policy and Practice;**

Detection methods for colistin resistance include antibiotic sensitivity testing, PCR, and whole genome sequencing, with newer techniques such as MALDI-TOF MS and real-time multiplex PCR are promising (Osei Sekyere, 2019). However, current phenotypic methods may not be suitable for detecting low-level resistance conferred by *mcr* genes, necessitating the development of new, targeted technologies for comprehensive detection of all colistin-resistant bacteria (Bardet & Rolain, 2018). Colistin resistance in Gram-negative bacteria has emerged as a major global concern, with several mechanisms identified. The *mcr-1* gene, in particular, has shown rapid spread throughout Africa and is primarily carried by IncHI2-type plasmids (Hassen et al., 2022). Surveillance and monitoring of colistin resistance are vital to prevent the spread of resistant bacteria and maintain the efficacy of this antibiotic (Masseron et al., 2019). The European Society of Clinical Microbiology and Infectious Diseases emphasizes the importance of

detecting and monitoring carbapenem-resistant strains, particularly those that are pan-resistant and resistant to polymyxins (Hu et al., 2023). Overall, the molecular characterization of colistin-resistant Gram-negative bacteria is essential for informing policy and practice in the fight against antimicrobial resistance. Continued research and surveillance efforts are required to address this global health threat effectively.

### **7.3 Future Research Directions;**

Studies have proven this resistance in several bacterial species worldwide.

The fact of its ability to pass on from one bacterium to another, between animals and humans; the *mcr1-10* genes were acknowledged as the major responsible factor for colistin resistance. Apart from *mcr* genes, some chromosomal genes, like *mgrB*, *PhoP-PhoQ*, *PmrA-PmrB*, and efflux pump have been considered as potential factors for colistin resistance. Other strategies such as the nano-based strategy, photodynamic therapy, CRISPRi based strategy, and Phage-based strategy, could be employed to fight drug-resistance and provide an option to manage this issue (Aghapour Z. et al., 2019; Lomonaco S. et al., 2018; Skov R.L., et al., 2016; Sharma D. & Fluopsin C., 2020). An integrated multisectoral approach can also join in the fight against colistin resistance, better integration of human health, veterinary, and environment (Andersson D. I. et al., 2014; Robinson T.P. et al., 2016; Larsson D.G.J. et al., 2014). Countries and international agencies have included a One Health perspective within their action plans to address antimicrobial resistance. These actions include improvements in antimicrobial use regulation and policy, surveillance, stewardship, infection control, sanitation, animal husbandry, and alternatives to antimicrobials. These improvements aim to preserve the effectiveness of antimicrobials that are important for human medicine (Hoelzer K. et al., 2017; Tang K.L. et al., 2017; Scott A.M. et al., 2018). Different surveillance systems were established in European countries to check for colistin consumption and the emergence of resistance (Borck Høg K et al., 2017; Veldman K. et al., 2019; SWEDRES/SVARM, 2021). There are also different directions for further research on colistin. At a purely preclinical level, one is the verification of the stability of the intravenous solution and nebulization solution at relevant concentrations and conditions corresponding to clinical practice. Compatibility with other drugs should also be tested as well as mapping the current microbiological and susceptibility testing practices and monitoring colistin resistance development (Rychlíčková, J. et al., 2023). More pharmacokinetic and clinical-outcome data in critically ill patients receiving colistin at a standardized dosage should also be evaluated, including the loading dose. These can help us to estimate the plasma concentration time curve of colistin without extensive sampling and quantifying changes induced by extracorporeal methods. Finally, precise definition of colistin toxicity predictors would be helpful (Rychlíčková, J. et al., 2023).

**Disclaimer (Artificial intelligence)**

**Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.**

## REFERENCES

1. Cox D. *Antibiotic resistance: the race to stop the silent tsunami facing modern medicine.* *The Guardian.* 2015 Access date: 17 March 2018. Available from: <https://www.theguardian.com/society/blog/2015/aug/21/antibiotic-resistance-the-race-to-stop-the-silent-tsunami-facing-modern-medicine>.
2. M. Exner, S. Bhattacharya, B. Christiansen, J. Gebel, P. Goroncy-Bermes, P. Hartemann, et al. *Antibiotic resistance: what is so special about multidrug-resistant Gram-negative bacteria?* *GMS Hyg Infect Control*, 12 (2017), pp. 1-24
3. M. Reale, A. Strazzulla, A. Quirino, C. Rizzo, V. Marano, M.C. Postorino, et al. *Patterns of multi-drug resistant bacteria at first culture from patients admitted to a third level University hospital in Calabria from 2011 to 2014: implications for empirical therapy and infection control.* *Infez Med*, 25 (2017), pp. 98-107
4. I. Martin-Loeches, E. Diaz, J. Valles. *Risks for multidrug-resistant pathogens in the ICU.* *Curr Opin Crit Care*, 20 (2014), pp. 516-524
5. T.P. Van Boeckel, S. Gandra, A. Ashok, Q. Caudron, B.T. Grenfell, S.A. Levin, et al. *Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data.* *Lancet Infect Dis*, 14 (2014), pp. 742-750
6. WHO. *Antimicrobial Resistance: Global report on surveillance 2014.* [Accessed on 12 April 2020]. Available at: <https://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/>
7. World Bank. *Drug-resistant infections: a threat to our economic future.* *World Bank Rep.* 2016 [Google Scholar]
8. Founou RC, Founou LL, Essack SY. *Clinical and economic impact of antibiotic resistance in developing countries?: A systematic review and meta-analysis.* *PLoS One.* 2017;12:e0189621. doi: 10.1371/journal.pone.0189621. [DOI] [PMC free article] [PubMed] [Google Scholar]
9. Sharma D, Patel RP, Zaidi STR, Camerino GM, Aldo B, Moraes LA. *Interplay of the quality of ciprofloxacin and antibiotic resistance in developing countries.* *Front Pharmacol.* 2017;8:546. doi: 10.3389/fphar.2017.00546. [DOI] [PMC free article] [PubMed] [Google Scholar]
10. NCDC. *Antimicrobial use and resistance in Nigeria.* 2017. [Accessed on 4 March 2020]. Available at: [http://www.ncdc.gov.ng/themes/common/docs/protocols/56\\_1510840387.pdf](http://www.ncdc.gov.ng/themes/common/docs/protocols/56_1510840387.pdf).
11. Olowo-okere A, Abdullahi MA, Ladidi BK, et al. *Emergence of metallo-β-lactamase producing Gram-negative bacteria in a hospital with no history of carbapenem usage in*

northwest Nigeria. *Ife J Sci.* 2019;21:323–31. doi: 10.4314/ijss.v21i2.6. [DOI] [Google Scholar]

12. Yusuf I, Arzai AH, Haruna M, Sharif AA, Getso MI. Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria. *Braz J Microbiol.* 2014;45:791–8. doi: 10.1590/S1517-83822014000300005. [DOI] [PMC free article] [PubMed] [Google Scholar]
  - a. Nigerian Bureau of Statistics. Demographic statistics bulletin. 2017. [Accessed on: 12 April 2020]. Available at [www.nigerianstat.gov.ng](http://www.nigerianstat.gov.ng).
13. EUCAST. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. 2019. [Accessed on month 12 April 2020]. Available at: [www.eucast.org/mic\\_distributions\\_and\\_ecoffs](http://www.eucast.org/mic_distributions_and_ecoffs).
14. EUCAST. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and / or epidemiological importance. 2019. [Accessed on 12 April 2020]. Available at: [https://www.eucast.org/resistance\\_mechanisms](https://www.eucast.org/resistance_mechanisms).
15. Bakour S, Garcia V, Loucif L, et al. Rapid identification of carbapenemase-producing Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using a modified Carba NP test. *New Microbes New Infect.* 2015;7:89–93. doi: 10.1016/j.nmni.2015.07.001. [DOI] [PMC free article] [PubMed] [Google Scholar]
16. Magiorakos A, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;18:268–81. doi: 10.1111/j.1469-0691.2011.03570.x. [DOI] [PubMed] [Google Scholar]
17. Kadri SS, Adjemian J, Lai L, et al. Difficult-to-treat resistance in Gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. *Clin Infect Dis.* 2018;67:1803–14. doi: 10.1093/cid/ciy378. [DOI] [PMC free article] [PubMed] [Google Scholar]
18. Tacconelli E, Sifakis F, Harbarth S, et al. Surveillance for control of antimicrobial resistance. *Lancet Infect Dis.* 2018;18:e99–106. doi: 10.1016/S1473-3099(17)30485-1. [DOI] [PubMed] [Google Scholar]
19. Bostanghadiri, N., Narimisa, N., Mirshekar, M., Zankbar, L. D., & Taki, E. (2024). Prevalence of colistin resistance in clinical isolates of *Acinetobacter baumannii*: a systematic review and meta - analysis. *Antimicrobial Resistance & Infection Control*, 13(24), 1–17. <https://doi.org/10.1186/s13756-024-01376-7>
20. Dadashi, M., Sameni, F., Bostanshirin, N., & Yaslianifard, S. (2022). Global prevalence and molecular epidemiology of mcr -me diate d colistin resistance in *Escherichia coli* clinical isolates : a systematic review. *Journal of Global Antimicrobial Resistance*, 29, 444–461. <https://doi.org/10.1016/j.jgar.2021.10.022>

21. Folorunso, J. B., Bakhrebah, M. A., Garout, M., & Alfouzan, W. A. (2022). *Global Prevalence of Colistin Resistance in Klebsiella pneumoniae from Bloodstream Infection : A Systematic Review and Meta-Analysis*. *Pathogens*, *11*(1092), 1–12.
22. Bostanghadiri, N., Narimisa, N., Mirshekar, M., Zankbar, L. D., & Taki, E. (2024). *Prevalence of colistin resistance in clinical isolates of Acinetobacter baumannii : a systematic review and meta □ analysis*. *Antimicrobial Resistance & Infection Control*, *13*(24), 1–17. <https://doi.org/10.1186/s13756-024-01376-7>
23. Dadashi, M., Sameni, F., Bostanshirin, N., & Yaslianifard, S. (2022). *Global prevalence and molecular epidemiology of mcr -me diate d colistin resistance in Escherichia coli clinical isolates : a systematic review*. *Journal of Global Antimicrobial Resistance*, *29*, 444–461. <https://doi.org/10.1016/j.jgar.2021.10.022>
24. Narimisa, N., Keshkar, A., Dadgar-zankbar, L., Bostanghadiri, N., & Far, Y. R. (2024). *Prevalence of colistin resistance in clinical isolates of Pseudomonas aeruginosa : a systematic review and meta-analysis*. *Frontiers in Microbiology*. <https://doi.org/10.3389/fmicb.2024.1477836>
25. Lutgring, J. D., Kim, A., Campbell, D., Karlsson, M., Brown, A. C., & Burd, E. M. (2019). *Evaluation of the MicroScan Colistin Well and Gradient Diffusion Strips for Colistin Susceptibility Testing in Enterobacteriaceae*. *Journal of Clinical Microbiology*, *57*(5). <https://doi.org/10.1128/JCM.01866-18>
26. Irek, E. O., Amupitan, A. A., Obadare, T. O., & Aboderin, A. O. (2018). *A systematic review of healthcare-associated infections in Africa: An antimicrobial resistance perspective*. *African Journal of Laboratory Medicine*, *7*(2). <https://doi.org/10.4102/AJLM.V7I2.796>
27. Rychlíčková, J.; Kubičková, V.; Suk, P.; Urbánek, K. *Challenges of Colistin Use in ICU and Therapeutic Drug Monitoring: A Literature Review*. *Antibiotics* 2023, *12*, 437.
28. <https://doi.org/10.3390/antibiotics12030437>.
29. Iwu CJ, Jordan P, Jaja IF, et al. *Treatment of COVID-19: implications for antimicrobial resistance in Africa*. *Pan Africa Med J*. 2020;35:119.
30. Madubuike Umunna Anyanwu, Ishmael Festus Jaja, James Wabwire Oguttu, Chinwe Juliana Jaja, Kennedy Foinkefu Chah & Vincent Shodeinde Shoyinka (2021) *Is Africa ready for mobile colistin resistance threat?*, *Infection Ecology & Epidemiology*, *11*:1, 1962781, DOI: 10.1080/20008686.2021.1962781
31. Nuhu, T., Olawale, S., & Raji, M. (2024). *Evaluation of Co-production of Colistin Resistance and ESBL Genes among Gram-negative Clinical Isolates from Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria*. *UMYU Journal of Microbiology Research (UJMR)*, *9*, 134–146. <https://doi.org/10.47430/ujmr.2491.015>

32. Osei Sekyere, J. (2019). *Mcr colistin resistance gene: A systematic review of current diagnostics and detection methods*. *MicrobiologyOpen*, 8(4), e00682. <https://doi.org/10.1002/mbo3.682>
33. Iroha, C., Ugwu, M., Esimone, C. O., Iroha, I., Fa, I., & Ejikeugwu, C. (2023). *MOLECULAR CHARACTERIZATION OF ESBL AND COLISTIN RESISTANCE GENES AMONG CLINICAL ISOLATES OF GRAM-NEGATIVE BACTERIA IN A TERTIARY HOSPITAL IN ABAKALIKI, NIGERIA*. *International Journal of Pharmaceutical Sciences and Research*, 14, 3063. [https://doi.org/10.13040/IJPSR.0975-8232.14\(6\).3063-69](https://doi.org/10.13040/IJPSR.0975-8232.14(6).3063-69)
34. Kim, S., Woo, J. H., Kim, N., Kim, M. H., Kim, S. Y., Son, J. H., Moon, D. C., Lim, S.-K., Shin, M., & Lee, J. C. (2019). *Characterization Of Chromosome-Mediated Colistin Resistance In Escherichia coli Isolates From Livestock In Korea*. *Infection and Drug Resistance*, 12, 3291–3299. <https://doi.org/10.2147/IDR.S225383>
35. Mondal AH, Khare K, Saxena P, Debnath P, Mukhopadhyay K, Yadav D. *A Review on Colistin Resistance: An Antibiotic of Last Resort*. *Microorganisms*. 2024 Apr 11;12(4):772.