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Prevalence and Molecular Characterization of Colistin Resistance in Gram-negative Bacteria from Nigeria Hospitals: A Systematic Review

Samuel Chukwuemeka Okenwa [a], Joel Chekwube Ugwuezea [b*], Chika Augusta Ekweozor [a], Ifunanya Genevieve Anunwa [a], Jane Chidera Nwachukwu [c], Favour Mmesoma Nwankwo [a], Chukwuemeka Chidindu Njoku [d], Chidimma Elizabeth Mbakamma [a], Ebere Edith Onyishi [a], Ifeanyi Joseph Obiekwe [e] and Joy Ulonwadi Ajuonu [a]

[a] Faculty of Pharmaceutical Science, University of Nigeria Nsukka, Nigeria.
[b] Radiology Department, University College Hospital, Ibadan, Oyo, Nigeria.
[c] College of Medicine, University of Nigeria Teaching Hospital, Enugu, Nigeria.
[d] Department of Obstetrics and Gynaecology, Nnnamdi Azikiwe University Teaching Hospital, Anambra, Nigeria.
[e] Department of Biochemistry, Faculty of Biological Science, University of Nigeria, Nsukka, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: ugwuezeajoel123@gmail.com;*

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Review Article

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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.

Keywords: Molecular characterization; antibiotic resistance; public health; gram-negative bacteria.

1. INTRODUCTION

1.1 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, Nishino & Zhang, 2015). "Deaths from antibiotic resistance is far outstripping even those of epidemics such as Ebola and COVID-19" (David et al., 2021). "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" [1]. "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" [2]. "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" (Hartemann et al., 2017). "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" [3]. "Infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) have been reported to have increased significantly worldwide in recent years [4,5,6]. 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., 2017).

"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 [7,8].

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, 2022).

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 20181. In low- and middle-income countries (LMICs), antibiotic usage increased by 76% and is projected to continue rising by 2030 [9,10].

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 (Busayo, 2020) [11-14,15]. 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 (Nabti, 2020).

"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" (Olayinka, 2020). "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 [1].

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 [16]. 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).

1.2 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 [17,18,19].

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 [20-27].

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.

Rationale for studying Colistin in Nigeria: Colistin is rarely used in hospitals and clinics in Nigeria [28]. 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.

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

1.5 Inclusion Criteria

Studies published in English.

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

Studies focusing on patients with bacterial infections.

1.6 Exclusion Criteria

Studies focusing on viral infection cases.

Studies conducted in pediatric populations.

Studies not reporting healthcare expenditure of quality of life outcomes.

Methodology:

Systematic literature review:

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

Hand searching of relevant journal.

2. EPIDEMIOLOGY OF COLISTIN RESISTANCE

Global prevalence of colistin resistance: Bostanghadiri et al conducted a meta-analysis showed that the is a decrease in colistin reistsnace prevalence amongst isolates of Acinetobacter baumannii, the organism implicated for infections globally for the past two decades, though some countries have witnessed an incerase 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 regiosn where there is a heavy use of polymyxins liqe Qatar, Israel and france. This is avery disturbing fact as colistin is among the last line antibiotic therapy for extensively drug-resistant strains of Acinetobacter baumannii [29]. Folorunso et al. [30], 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 [31]. 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 [29]. 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 [31]. 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 [29]. A study conducted by Narimisa et al showed a 1% global resistance of all isolates of P. aeruginosa to colistin. Patientrs with cystic fibrosis showed a 7% resistance to colistin which is the highest among the diseases examined. It also observed in increase in global colistin resistance from 2% to 5% [31].

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 (Omoruyi et al., 2023). 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 [32]. 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 (Omoruyi et al., 2023). 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 (Ngbede 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 ($p = 0.039$).

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 baumanii complex isolates was <80% to all antibiotics apart from colistin [32]. Globally, the hospital environment faces a common challenge of HAIs (Hospital Acquired Infections) 87% 0f HAIs are caused by gram negative organisms [33], Klebsiella spp., S aureus, Acinetobacter spp., and E. coli are the most implicated bacterial causing Hospital acquired infections in Africa (Bamford, 2015). 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 [34].

The use of colistin in the prevention, control and treatment of infection in animals may be associated in the emergence of its resistance [35] 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 [35].

3. 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 [36]. 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 [36]. 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 [37]. 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 [38]. 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 [39]. 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 [39]. Resistance mechanisms are presumed to be linked to chromosomal mutation untransferable via horizontal gene transfer [36]. 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 [40].

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 [41-43].

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 (Hussein 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 [44].

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 [45-47,48].

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 [49].

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 (Carrol et al., 2019).

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 (Carrol et al., 2019).

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 (Carroll et al., 2019).

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. 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 (NEB5α) 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. [44], 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.

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 [50]. Overexpression in these pumps are recognised to be linked to a resistance against drugs [51]. Efflux pumps reduces the drug concentration without modification of the antibiotic [52]. The efflux pump mechanisms are caused by mutations in the regulatory genes [53,54]. 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 [55,56,57]. The RND is found in Gram-negative bacteria, wwhile the others are found in both Gram-positive and negative bacteria [58]. The colistin resistance mechanisms in gram-negative bacteria have been summarized in many previous reviews [59,60]. 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) [39]. A study on A. hydrophila suggests that the outer membrane lipoprotein-encoding gene, MlaA, may be associated with high levels of colistin resistance [39]. Also, Zeng et al. found that the RpoE stress system mediated colistin resistance in E. coli without disturbing the lipid A profile [61]. The diversity of colistin resistance mechanisms was discoverd by colistin-degrading proteases [62]. Colistin, as a cationic polypeptide compound, acts mainly on the outer membrane of bacteria carrying a negative charge [63]. 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 [64]. Efflux pumps, such as the KpnEF, AcrAB and Sap proteins, have been reported in Enterobactericeae. By activation of these pumps, resistance to colistin is increased [65,64]. 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 [66].

4. 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 [67]. 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 [68]. Scientists have used primers of the original study [36], or designed their own primers for standard PCR [69,70,71,72,63,73], or RTPCR, based on SYBR Green assays [74,70,75], TaqMan probe [76,77,71,78], or other FAM-labelled probe [79,80,81,82] or HEX-labelled probe [83]. Xavier et al. designed primers to screen mcr-2 [84]. Some designed their own primers for standard PCR (Sun J. et al., 2017) [85], and a study developed a TaqMan assay for qPCR (Roschanski, N. et al., 2017). These primers were designed for detecting mcr-3 [9], mcr-4 [86], and mcr-5 genes [87] 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; [88]. PCR also detect plasmid carrying mcr genes [89]. This sequence may form a composite transposon that can potentially move as one complete unit [63,90,91]. This insertion sequence appears to be a key component of the mobilome, and its presence is not systematic [83]. Li et al. identified the ability of the Tn6330 transposon to generate circular ISApl1-mcr-1-orf [92]. Specific primers were developed to screen the upstream presence of this IS transposon by PCR and Sanger sequencing [93,94,95]. Others have also designed their own system to directly screen on plasmid carrying mcr-gene type IncX4 [79,96].

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 [97] 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 et al. (2022), 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 [98]. According to [99] 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 [100]. 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 [100].

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).

5. 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 (Barnes et al., 2019). 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 [101-104]. 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 Enterobacteriaceaea [105].

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 bacterials [106].

Combination of colistin with other antibiotics are popularly called combinatorial therapy [107] 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 [108] the mcr (mobilized colistin resistance) gene is the strain responsible for colistin resistance [109] also, a combination of colistin and tigecycline exhibited substantial antimicrobial activity against E.coli habouring mcr -1 in contrast to when the antibiotics were used independently, even in higher doses [110].

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 [111]. 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 anti depressants (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, chloropromazine or levopromazine against multi drug-resistant A.baumannii, E.coli and K. pneumoniae [112]. The best approach to combat AMR remains public awareness on the dangers of antibiotics abuse [109].

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 [113]. 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 [114]. Colistin is used as the last alternative antimicrobial against MDR and PDR (pan-drug-resistant) Gram-negative infections [115], (Li J. et al., 2006) [116]. Different bacterial species have developed resistance due to the inappropriate use of colistin [117-122,41-43,45-47,101-103]. Prevention strategies play a crucial role in the management of colistin-resistant genes. These strategies include: genotyping and rapid diagnosis, survillence culture, infection control measures and antimicrobial stewardship progamme [123]. 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 [124]. 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 [113].

6. 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 [125]. 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 [126]. Phenotypic methods include agar-based media, MIC-determiners, and rapid colorimetric tests, while molecular methods encompass PCR, LAMP, and whole-genome sequencing [127]. 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 [100]. Current research aims to develop more efficient, sensitive, and specific diagnostics to address the growing concern of colistin resistance [128]. 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 [129]. In the clinical setting, MALDI-TOF MS has emerged as a reliable tool for detecting colistin-resistant pathogens, particularly Gram-negative bacteria [87] However, there is a lack of consensus on the methodology for colistin susceptibility testing, leading to challenges in accurately identifying resistant strains [130]. In Gram-negative bacteria, mechanisms of resistance may involve genomic mutations that decreases membrane permeability, and also the exchange of carbapenemase genes on plasmids [126] Moreover, the occurrence of carbapenem-resistant strains further complicates the treatment of infections caused by multidrug-resistant Gram-negative bacteria [130,131]. Overall, the molecular characterization of colistin-resistant Gram-negative bacteria presents significant challenges in current detection methods [122]. 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 [125]. 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 faciliting 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 other 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 [132] 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 [60]. 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 [113,133,68,134]. Collaboration between the various health and veternairy sector to prevent colistin resistance is of importance also [135,136,137].

7. 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 [138]. 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 [67]. 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 [139]. Surveillance and monitoring of colistin resistance are vital to prevent the spread of resistant bacteria and maintain the efficacy of this antibiotic [140]. 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 [141]. 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 [113,133,68,134]. An integrated multisectoral approach can also join in the fight against colistin resistance, better integration of human health, veterinary, and

environment [135,136,137]. Countries and international agencies have included a One Health perspectivewithin 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 [142,143,144]. Different surveillance systems were established in European countries to check for colistin consumption and the emergence of resistance [145,138]. 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 [144]. 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 quantifing changes induced by extracorporeal methods. Finally, precise definition of colistin toxicity predictors would be helpful [142].

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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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