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Research Article
Detection and Dissemination of MCR-1 Colistin Resistance Gene in a Hospital Setting in Abakaliki, Nigeria

[1]Chidinma Iroha, [2]Malachy Ugwu, [3]Ifeanyichukwu Iroha, [2]Charles Esimone and [4]Chika Ejikeugwu

[1]Department of Pharmacy, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria
[3]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria
[4]Department of Pharmaceutical Microbiology and Biotechnology, Enugu State University of Science and Technology, Agbani, Nigeria

Abstract

Background and Objective: Colistin is a last-line antibacterial agent used in clinical medicine. In this study, the dissemination of bacteria harbouring *mcr-1* colistin resistance genes in a hospital setting was investigated. **Materials and Methods:** A total of 500 clinical samples from urine, wound, High Vaginal Swab (HVS), sputum and stool were bacteriologically investigated for the isolation and identification of Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using standard microbiology techniques. The isolated bacteria were further screened for colistin resistance phenotypes and the detection of the colistin resistance gene, *mcr-1* gene using the Polymerase Chain Reaction (PCR) technique. **Results:** Colistin resistance was phenotypically detected in *E. coli* isolates from urine (8.2%), wound (4.8%), HVS (4.6%), stool (2.6%), sputum (1%), *K. pneumoniae* from urine (2.8%), HVS (3.6%), stool (2.2%), sputum (3.8%), and *P. aeruginosa* from urine (2%), wound (3.8%), stool (1%), sputum (1.6%). Among the colistin-resistant isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa*, only 1 (0.6%) *E. coli* isolate and 2 (3.2) isolates of *P. aeruginosa* harboured the *mcr-1* gene. None of the colistin-resistant *K. pneumoniae* isolates was confirmed by PCR to carry the *mcr-1* gene that mediates bacterial resistance to colistin. **Conclusion:** The growing prevalence of colistin resistance in bacteria warrants urgent steps to preserve the therapeutic efficacy of this antibacterial agent. This study points to the likely spreading of bacteria harbouring the *mcr-1* gene in hospitals. The resistance of bacterial pathogens to colistin and other antibacterial agents could jeopardize antibiotic therapy if left unchecked.

Key words: Antimicrobial resistance, *mcr-1* genes, Gram-negative bacteria, colistin resistance, bacterial pathogen, sputum, polymyxin E

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Corresponding Author: Chika Ejikeugwu, Department of Pharmaceutical Microbiology and Biotechnology, Enugu State University of Science and Technology, Agbani, Nigeria Tel: +2347081775676

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The evolution and nosocomial transmission of antibiotic resistance (ABR) in the human population presents a formidable challenge for the public health sector globally and a greatest public health threat. When ABR occurs, antibiotics become less effective and bacteria fail to be killed or inhibited by (even) lethal concentrations of the drug. Long hospitalization, severe illness and morbidity, as well as mortality, are the major fallouts of the evolution and transmission of ABR in a healthcare (nosocomial) setting. A recent study estimates that by the year 2050, 10 million deaths per year will be attributable to antibiotic resistance worldwide[1]. The rising cases of ABR across the globe are gradually pushing the world into a post-antibiotic era where antibiotics will no longer be effective to fight the prevalent infectious diseases in human populations[2-6]. According to Yin *et al.*[7] the inappropriate use of antibiotics in hospitals has been reported in many Low- and Middle-Income Countries (LMICs). Resistant microorganisms thrive globally and are transmitted to and from healthy humans, animals and the environment, particularly in many LMICs settings like Nigeria[3,8]. More worrisome is the fact that antibiotics are readily available Over-The-Counter (OTC) even without prescription in Nigeria, for both clinical and non-clinical purposes. A scenario like this portends grave danger for the developing healthcare system of the country, as well as encourages the evolution and transmission of AMR pathogens which causes the antibiotics used to treat infections to become less effective. Colistin has increasingly been used as a "last-chance" therapeutic antibacterial agent due to its broad spectrum of activity. Its clinical usage is a result of the persistent rise in ABR noticed in bacteria producing some high-level antibiotic degrading enzymes including metallo beta-lactamase, AmpC enzymes and extended spectrum beta lactamase[3]. Also known as polymyxin E, colistin is a typical example of antimicrobial peptide with improved antimicrobial properties when compared to conventional antibiotics which are currently losing their efficacy against most drug-resistant bacteria. They are used clinically to treat bacterial infections in cystic fibrosis patients and multidrug-resistant infections[9]. But the clinical efficacy of colistin is being jeopardized by the emergence and transmission of colistin-resistant genes, particularly the *mcr-1* genes that encode for resistance in pathogenic bacteria harbouring them. The *mcr-1* gene is a colistin resistance gene that is horizontally transmissible and mediates bacterial resistance to colistin and its mechanism of action is that the *mcr-1* gene encodes a PEtN transferase that modifies lipid A to reduce anionic charge in the target bacteria[10], thereby sparking up processes that leads to the reduced susceptibility of the test pathogen to these AMPs (i.e., colistin and polymyxin B). Acquired resistance to colistin was previously linked to chromosomal mutations until recently when the first report of plasmid-mediated transfer of colistin-resistant genes in animals and human isolates of *Escherichia coli* and *Klebsiella pneumoniae* was documented in China[11]. Furthermore, higher rates of colistin resistance have been documented in isolates from food-producing animals and the environment across Nigeria[12,13]. According to Meurer *et al.*[14], the development of resistance to AMPs does not seem to the occur, if at all, at the rate of the resistance to conventional antibiotics. Nonetheless, it is critical to update the susceptibility profiles of bacterial pathogens to the AMPs (e.g., colistin) to keep a close watch and track the evolution and transmission of strains resistant to colistin-which is perhaps an important antibiotic currently used in the healthcare settings across the globe. The prevalence and transmission of bacterial pathogens harbouring the *mcr-1* gene in a hospital environment are a critical public health crisis because these pathogens are notably resistant to colistin, an important antibacterial agent and can be passed on to patients and visitors from where the resistant bacteria can spread to the community.

This study investigated the dissemination of *mcr-1* colistin resistance genes in a hospital setting in Abakaliki, Nigeria.

MATERIALS AND METHODS

Study area and ethics: The study was carried out at the Laboratory of Applied Microbiology Department, Ebonyi State University, Nigeria from January, 2021 to April, 2022. All the experimental protocols carried out in this study were approved by the Ethics and Research Committee of the State Ministry of Health, Ebonyi State, Nigeria (SMOH/ERC/042/21). All the methods were carried out in strict compliance with the guidelines of the World Medical Association Declaration of Helsinki on the principles for medical research involving human subjects and identifiable human material/data.

Collection and processing of clinical samples: Non-duplicate sputum (n = 100), urine (n = 100), wound (n = 100), high vaginal swab (n = 100) and stool samples (n = 100) were collected from out-going patients in a healthcare setting using sterile sample collection containers. All samples were bacteriologically processed in the Microbiology Laboratory of Ebonyi State University, Nigeria according to all relevant national and international guidelines.

Characterization of bacteria: Standard microbiology techniques including culture, microscopy, biochemical testing and colonial features of bacteria on selective culture media were used to process all clinical samples. The isolates from the clinical samples processed on cetrimide selective agar, Eosin methylene blue agar and MacConkey agar were bacteriologically and microscopically confirmed to be *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*. The molecular characterization of isolates was further determined using the 16S rRNA gene amplicon protocol and gene sequencing technique[10,11].

Phenotypic detection of colistin resistance: Resistance of the test bacteria isolates to colistin was first phenotypically determined using the E-Test Strip (AB, Sweden) for minimum inhibitory concentration (MIC) with Mueller Hinton (MH) agar plates aseptically swabbed with defined concentrations of the test bacteria equivalent to 0.5 McFarland turbidity standards. The MIC values were interpreted following the breakpoints of the approved standard of the Clinical Laboratory Standard Institute, CLSI[10,12-15]. All test plates were incubated at 37°C for 18-24 hrs. Bacterial isolates having a MIC$\geq$4 mg L^{-1} against colistin were inferred to be colistin-resistant and further screened for the presence of the *mcr-1* colistin resistance gene using PCR.

PCR detection of *mcr-1* gene: PCR technique was used to further screen all bacterial isolates with a MIC$\geq$4 mg L^{-1} against colistin for the presence of *mcr-1* genes which mediate colistin resistance in bacteria[10,11,13]. Briefly, bacterial DNA was extracted from the test isolates and purified using the Zymo Plasmid miniprep extraction kit (Epigenetics Company, USA). PCR analysis was performed on a 2720 thermal cycler (Applied Biosystems, Life Technologies, USA) with the right PCR conditions pre-installed and started for the gene amplification process using primers unique to *mcr-1* gene (F-5-CGGTCAGTCCGTTTGTTC-3 and R-5-CTT GGTCGGTCTGTAGGG-3) and synthesized and supplied by Inqaba Biotechnical Industries Ltd., (Inqaba Biotechnical Industries Ltd., South Africa). The final mix for the PCR analysis comprised 26.5 µL of the master mix containing 0.2 µL of Taq polymerase enzyme U μL^{-1}, 2.5 µL of 10X PCR buffer along with 2.5 µL $MgCl_2$, 1 µL of 10 pM from each of the forward and reverse primers, 2.5 µL of dNTPs MIX (2 Mm), 3 µL of DNA template (from the test isolates), 14.8 µL of nuclease-free water. A 100 bp DNA molecular marker was used as the positive control while the negative control was a PCR master mix containing distilled water. Gel electrophoresis of the PCR products was carried out in 1.5 % agarose gel for 2 hrs at 80 V and photographed in a UV transilluminator (Thermo Scientific, USA).

RESULTS

The prevalence of the isolated bacteria in the various clinical samples analyzed in this study was shown in Table 1. The bacterial strains isolated included *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, with more isolates recovered from the wound (15%), urine (18.2%), stool (14%) and sputum samples (12%). Out of the 500 clinical samples bacteriologically analyzed in this study, only 355 bacterial strains were isolated from the clinical samples investigated in this study (Table 1). The results of the phenotypic detection and prevalence of colistin resistance phenotypes from the various clinical samples investigated in this study was shown in Fig. 1.

Off all the isolates recovered from the various clinical samples investigated for colistin resistance using the phenotypic detection technique, *Escherichia coli* isolates (8.2%) from urine samples were the most prevalent colistin resistance phenotype (Fig. 1). This was followed by *E. coli* isolates (4.8%) recovered from wound samples and *E. coli* isolates (4.6%) recovered from high vaginal swab samples. The percentage prevalence of *K. pneumoniae* isolates that were positive for colistin resistance in sputum and HVS samples were 3.8% and 3.6% respectively (Fig. 1). All *K. pneumoniae* isolates from wound samples were found to be susceptible to colistin and did not express colistin resistance phenotype.

Table 1: Frequency of bacteria strain in clinical samples analyzed

		Bacterial strain							
		P. aeruginosa		*E. coli*		*K. pneumoniae*		Occurrence rate	
Sample	Number of sample	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Wound	100	21	4.2	35	7.0	19	3.8	75	15.0
Urine	100	10	2.0	58	11.6	23	4.6	91	18.2
High vaginal swab	100	0	0.0	31	6.2	27	5.4	58	11.6
Stool	100	14	2.8	37	7.4	19	3.8	70	14.0
Sputum	100	17	3.4	12	2.4	32	6.4	61	12.2
Total	500	62	12.4	173	34.6	120	24.0	355	71.0

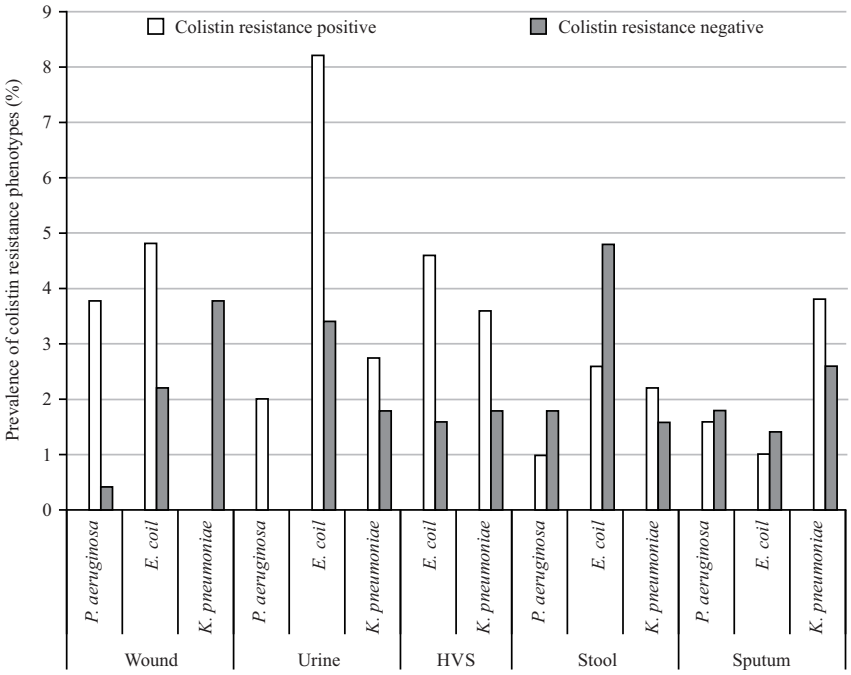

Fig. 1: Prevalence of colistin resistance phenotypes

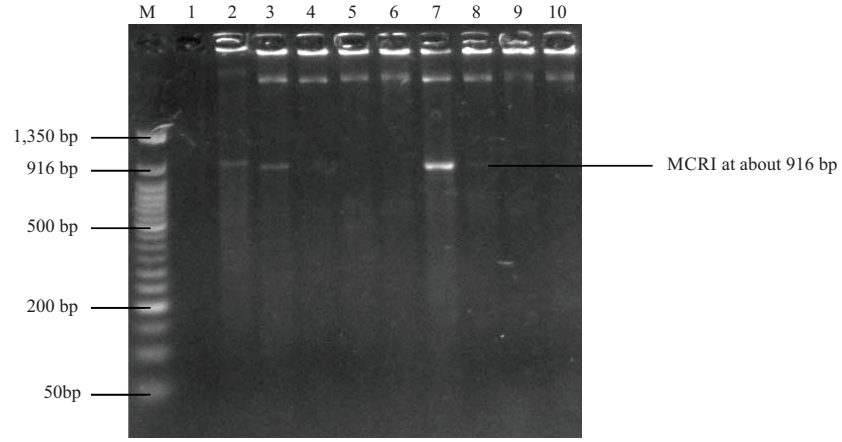

Fig. 2: Gel electrophoresis product showing detection and amplification of *mcr-1* colistin resistance gene in the test bacterial isolates investigated in this study

Lane M is the DNA ladder/molecular marker, lanes 1-9 show the various test samples investigated for the presence of the *mcr-1* gene, lanes 2, 3 and 7 show amplified products of *mcr-1* genes (with a base pair size of 916 bp) in colistin resistance phenotypes or isolates, lanes 1, 4, 5, 6, 8 and 9 were test samples with no amplified DNA product for *mcr-1* genes and lane 10 contains nuclease-free water as a negative control

The colistin resistance gene, *mcr-1* that mediates bacteria resistance to colistin was detected and confirmed by PCR technique in the test bacterial isolates of colistin-resistant phenotypes with a base pair size of 916 bp (Fig. 2). From this result, the *mcr-1* colistin resistance gene was detected in *E. coli* (0.6%) and *P. aeruginosa* strains (3.2%). The *mcr-1* gene that mediates colistin resistance was not detected in *K. pneumoniae* strains.

DISCUSSION

Antibiotic resistance is increasingly common and spreading globally, threatening our ability to treat some common bacterial infections. The widespread use of the antibiotics is a major contributing factor to the evolution and transmission of antibiotic-resistant bacteria in any environment[3]. This study investigated the dissemination of the

mcr-1 gene that mediates colistin resistance in a hospital setting. From current results, the resistance rate of colistin resistance was phenotypically detected first, in the range of 1-8.2% and these isolates were further investigated for the *mcr-1* gene using the PCR technique. It was discovered that *Escherichia coli* isolates from urine samples were the most prevalent colistin resistance phenotype (8.2%) detected in this study, followed by *E. coli* from wound samples (4.8%) and *E. coli* from high vaginal swab HVS samples (4.6%). In addition, colistin resistance phenotypes were also phenotypically detected in *K. pneumoniae* isolates from sputum samples (3.8%), *K. pneumoniae* from HVS (3.6%) and *K. pneumoniae* from urine samples (2.8%). For *P. aeruginosa* isolates, colistin resistance was phenotypically detected in strains from wound samples (3.8%), urine samples (2%) and sputum samples (1.6%). Elsewhere in South-South Nigeria, in Port Harcourt, colistin resistance was phenotypically reported in *E. coli* strains (8.2%) recovered from clinical samples[13]. This result was similar to ours in which colistin resistance was phenotypically reported in *E. coli* isolates recovered from urine samples (8.2%). A similar pattern of colistin resistance in Gram-negative bacteria of *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates from clinical samples of hospital origin was also reported and observed in other developing and developed countries - where resistance to colistin was implicated in some hospital-acquired infections[6,12,16-18]. These results confirmed that the prevalence of colistin resistance phenotypes in the different samples analyzed in this study contributes to the prevalence of colistin resistance in Gram-negative bacteria of hospital origin. Since the first isolation of the colistin resistance gene in China[10], the *mcr-1* gene that mediates colistin resistance in bacterial pathogens have continued to spread between human and animal microbiome, thus complicating the efficacy of antibiotic therapy and making infections persist. The *mcr-1* gene was only detected in *E. coli* (0.2%) and *P. aeruginosa* (3.2%) isolates while the *K. pneumoniae* isolates did not harbour the *mcr-1* gene even though they were phenotypically confirmed to exhibit resistance to colistin. In contrast to this present study, previous studies have reported high detection of the *mcr1* gene (5%) from clinical samples in Egypt[19]. However, a higher prevalence of the *mcr-1* gene has been previously reported in China in Gram-negative bacteria from animal and human samples[11]. The presence of pathogenic *E. coli* and *P. aeruginosa* harbouring *mcr-1* gene (that mediates resistance to colistin in bacteria) in the hospital environment portends serious public health risk because these pathogens could transfer the genes to other susceptible bacteria and thereby create an environment for the evolution of drug-resistant bacteria that will be difficult to treat with the available antibiotics. Though the current study reported a low prevalence of the *mcr-1* gene in *E. coli* and *P. aeruginosa* isolates, the detection of the *mcr-1* gene in these clinically important pathogens indicated the possible dissemination of the colistin resistance gene amongst hospital pathogens in this part of the world. This necessitates the need to introduce stringent detection and reporting protocol for colistin resistance in hospitals to prevent the further evolution and spread of these multidrug-resistant bacteria.

CONCLUSION

The overuse and underuse of antibiotics play a significant role in the development and spread of antibiotic resistance in the hospital environment. *mcr-1* gene is an important gene because it mediates bacterial resistance to colistin, an important antibiotic used for clinical applications. This preliminary study shows that the Gram-negative bacteria of hospital origin investigated in this work harboured the *mcr-1* gene in a low percentage. However, our study has indicated the possible dissemination of bacteria harbouring the *mcr-1* gene and is resistant to colistin, thus antibiotic therapy may be jeopardized. These pathogens harbouring the *mcr-1* gene could be a critical source of nosocomial infections caused by colistin-resistant bacteria. Further study is required to determine the actual disease burden of colistin resistance, particularly the rate of prevalence of the *mcr-1* gene dissemination in clinical samples and pathogens in the studied area.

SIGNIFICANCE STATEMENT

This study investigated the dissemination of colistin resistance bacteria within a hospital environment in Abakaliki, Nigeria. The most significant finding is the identification of the *mcr-1* gene that mediates colistin resistance in Gram-negative bacteria of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Bacteria harbouring colistin resistance genes are of public health importance because they could spread and cause infections that are difficult to treat with available antibiotics because of their multidrug resistance phenotypes. Further studies are required to identify the clonal spread of the resistance phenotypes to mitigate any possible disease outbreak due to these organisms.

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