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Molecular Detection of Mobilized Colistin Resistance (*mcr-1*) gene in *Escherichia coli* Isolates from Port Harcourt, Nigeria

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ABSTRACT: The emergence of plasmid borne colistin resistance in recent years has been problematic as a result of the potential for rapid dissemination through bacterial populations. This *mcr-1* mediated resistance has been reported from around the globe and active surveillance is essential to monitor the developing issue. This study set out to determine the occurrence of such strains in a group of 60 *Escherichia coli* isolates using DNA extraction and amplification techniques. Following molecular confirmation of the identities of the *E. coli* isolates based on the detection of *E. coli* specific 16sRNA gene fragments, phenotypic colistin resistance of isolates was determined and isolates were screened for the *mcr-1* gene using standard procedures. Of the 35 confirmed *E. coli* isolates, 60% were found to be colistin resistant, with a higher level of resistance noted among the non-clinical isolates. Plasmid mediated *mcr-1* resistance was however found to be present in only 8.6% of total isolates, making up 14.3% of the colistin resistant strains. This *mcr-1* mediated resistance was only noted in clinical isolates however. This detection of *mcr-1* mediated colistin resistance in *E. coli* isolates from Port Harcourt, Nigeria is worrisome as it could point at a looming epidemic of colistin resistance and hence the development of untreatable bacterial isolates. Further studies are essential to properly assess the scope and spread of this situation.

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Colistin, a member of the polymyxin family of antibiotics has in recent years been described by the World Health Organization as a human medicine of critical importance (WHO 2011). This antibiotic is currently considered a drug of last resort, used in the treatment of multidrug resistant Enterobacteriaceae. Over the years the introduction of new antibiotics has led to the subsequent and nearly immediate development of resistant isolates. This phenomenon has led to the current situation of a multidrug resistant global epidemic leading to the declaration of a state of emergency. One of the latest of such resistance which has increasingly been recognized, is the carbapenem resistance in Enterobacteriaceae described as carbapenem resistant Enterobacteriaceae (CRE).

First described in the late 2000s (Schwaber and Carmeli 2008), these resistant isolates are a further evolution of the notorious extended spectrum beta lactamases (ESBLs) producing bacteria. CRE resistance is as a result of the production of the carbapenemases enzymes which confer broad resistance to both carbapenems and most of the beta lactam antibiotics (Codjoe and Donkor 2018). Prior to the emergence of carbapenem resistance, carbapenem was considered a drug of last resort for ESBL producing bacteria, this role was therefore subsequently taken over by colistin. Similar to the trend over the years, the reintroduction of colistin into clinical practice led to the detection of colistin resistant bacteria in several key bacteria of clinical importance (Lim *et al.,* 2010).Subsequently, detection of colistin resistance has since been made, with resistance of up to 25% reported and a general worldwide prevalence of about 10%. Some studies however also went on to report an association between this resistance and specific regions (Giske 2015, Al-Tawfiq*et al.,* 2017).

Prior to 2016, colistin resistance was generally chromosomal mediated and hence associated with vertical transmission and a slow rate of evolution. This resistance was thought to involve the modification of bacterial lipopolysaccharide due to an upregulation of PhoP-PhoQ, as well as changes in the *mcrB* gene.

The colistin resistance story changed in 2016 with the first documentation of plasmid-mediated colistin resistance (Liu *et al.,* 2016). This was first described in China from *Escherichia coli* isolated from animals

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mediated by the *mcr-1* gene which encodes an enzyme belonging to the phospohethanolamine transferase family. Plasmid encoded colistin resistance is worrisome as it opens the possibility of horizontal gene transfer and hence a more rapid spread of this resistance through bacterial populations.

Though the initial report of this plasmid resistance noted a high association with animals, a SENTRY antimicrobial surveillance program published in 2016 which screened 390 clinical *E. coli* and *Klebsiella pneumonia* isolates previously noted as having MICs of ≥4µg/ml of colistin and 314 CRE, found 19 positive for *mcr-1*.

All positive isolates were *E. coli*, and had been obtained from 10 different countries (Castanheira *et al.,* 2016). A 2017 reported noted that*mcr-1* plasmid mediated colistin resistance has been detected in over 36 countries, three of which were African countries, with a single study from Nigeria (Al-Tawfiq *et al.,* 2017, Olaitan *et al.,* 2016).As this is a developing situation, surveillance is crucial to monitor the rate of development and dissemination of this resistance. This study therefore set out to assess a group of *E. coli* isolates for the presence of the *mcr-1* gene.

MATERIALS AND METHODS

Bacterial Isolates: A total of sixty *E. coli* isolates were used in this study (30 clinical isolates, 30 non-clinical), the identities of which were determined using standard biochemical tests (Cheesbrough 2000, Cowan and Steel 1985).

DNA Extraction: Bacterial DNA extraction was carried out using the standard boiling method (Oliveira *et al.,* 2014). This involved boiling of pure bacterial colonies in 100µL of molecular grade water for 5 min, followed by centrifugation (10,000g for 5min) which leaves bacterial DNA suspended in the supernatant.

Molecular confirmation of E. coli identity: Molecular confirmation of isolates as *E. coli* was based on the detection of *E. coli* specific 16s rRNA gene fragment using the Ec16 primer pair F 5′-GACCTCGGTTTAGTTCACAGA-3′ and R 5’-CACACGCTGACGCTGACCA-3′and standard amplification protocols as previously described (Islam *et al.,* 2016).

Screening for mcr-1 gene: Screening for *mcr-1* gene was carried out as previously described (Liu *et al.,* 2016) and simply involved the detection of the *mcr-1* gene fragment using the CLR primer pair: F 5′-GACCTCGGTTTAGTTCACAGA-3’ and R 5’-CACACGCTGACGCTGACCA-3’ and standard amplification protocols.

Antimicrobial susceptibility testing and screening for colistin resistance: Antimicrobial susceptibility testing was then carried out using the standard Kirby Bauer technique (Bauer 1966, NCCLS 2000). The commercial disc used contained ceftazidime, cefixime, ofloxacin, augmentin, nitrofurantoin, ciprofloxacin, cefuroxime and gentamicinantibiotics. Screening for colistin resistance was determined by analyzing the ability of isolates to grow at colistin concentrations of 2µg/ml in line with the EUCAST standard, which describes colistin resistant bacteria as isolates exhibiting a ≥ 2µg/ml MIC (Eucast 2016, Newton-Foot *et al.,* 2017).

RESULTS AND DISCUSSION

Following molecular confirmation, only 35 of the isolates were confirmed to be *E. coli* based on the presence of the *E. coli* specific 16s rRNA gene fragment, with a higher percentage of this comprised of the non-clinical isolates (Table 1).

Screening for colistin resistance: The general screen for colistin resistance found 60% of confirmed *E. coli* isolates to be colistin resistant. A higher percentage of this resistance was however found represented among the non-clinical isolates (Table 2).

Screening for mcr-1 colistin resistance: From the 35 confirmed *E. coli* isolates, the *mcr-1* gene was found to be present in 8.6% of isolates. These isolates made up 14.3% of the total colistin resistance though colistin resistance mediated by the *mcr-1* gene was found to occur only in the clinical isolates U3, U7 and U12.

C*haracteristics of mcr-1 mediated colistin resistant isolates*: These *mcr-1* mediated colistin resistant isolates were found to exhibit a similar range of susceptibilities. All of the three isolates were resistant to Augmentin, Ceftazidime and Cefuroxime, but susceptible to gentamicin, ofloxacin, nitrofurantoin and ciprofloxacin. U7 and U12 were additionally resistant to Cefixime.

The recent detection of the *mcr-1*plasmid mediated colistin resistance opened up the possibility of a new threat to the current war against drug resistance development. Though initially detected in animal strains of *E. coli*, *mcr-1* has since been reported in various clinical strains from around the globe and surveillance is crucial to monitoring the developing situation.

Table 1: Genotypic and Phenotypic Correlation of Isolate Identity

	Clinical	Non-Clinical
Molecular Confirmation of *E coli*	13 (43.3%)	22 (73.3%)

Table 2: Distribution of colistin resistance among isolates

	Clinical (n = 13)	Non-Clinical (n = 22)	Total (n = 35)
Colistin resistant isolates	6 (46.2%)	15 (68.2%)	21 (60%)

Prior to the detection of the *mcr-1* plasmid borne colistin resistance, colistin resistance had been generally described. In this present study, a general colistin resistance rate of 60% was noted. This was much higher than reports which described resistance rates ranging from 0.47% to14.3%, (Kluytmans-van den Bergh *et al.,* 2016, Luo *et al.,* 2017, Rossi *et al.,* 2017, Chan *et al.,* 2018, Del-Bianco *et al.,* 2018, Yoon *et al.,* 2018). The colistin resistance rate detected in this study was even still higher than studies which reported 'high rates' of colistin resistance (Nachimuthu *et al.,* 2016, Huang *et al.,* 2017, Alba *et al.,* 2018). These studies had reported rates ranging from 24.3% to 28.7%.Some of these studies describing high rates of colistin resistance had noted them in non-clinical isolates. These higher rates have been thought to result from a use of colistin in farming as a growth hormone whereby the drug is used in low concentrations to alter the flora of the animals leading to improved nutrient uptake (Al-Tawfiq *et al.,* 2017). This study similarly noted a higher rate of colistin resistance in the non-clinical isolates as opposed to the clinical isolates.

Table 3: Antibiogram of colistin resistant isolates

Isolate	Antibiogram
U3	AUG-CAZ-CRX
U7	AUG-CAZ-CFM-CRX
U12	AUG-CAZ-CFM-CRX

Following the initial report of *mcr-1* plasmid mediated colistin resistance; active surveillance has taken place to properly understand the scope of the problem. Overall prevalence rates ranging from 0.14% to 2.08% have been widely reported, with majority of reports showing a less than 1% prevalence rate (Luo *et al.,* 2017, Saavedra *et al.,* 2017, Cao *et al.,* 2018, Chan *et al.,* 2018, Del-Bianco *et al.,* 2018). These rates are much lower than the 8.3% overall prevalence reported in this study. The single outlier study reporting a prevalence value out of this range (4.7%) was a report from Thailand (Eiamphungporn *et al.,* 2018). Focusing specifically on the rate of colistin resistance linked with *mcr-1*, studies went on to report rates ranging from 2.3% to 52.5%. These studies differed in their source of isolates, geographical location and time frame covered. One study which reported an 83% rate of colistin resistance linked with *mcr-1* made these findings in non-clinical isolates rather than clinical isolates (Newton-Foot *et al.,* 2017). The relatively low rate of16.8% detected in this study might perhaps point at a lack of dissemination of this gene in this locale. Alternatively, the relatively low rate might be

An indication of the lack of extensive sampling carried out. The limited number of isolates analyzed is a major limitation of this study. The results of this study could therefore serve as preliminary data which would require more extensive, possibly hospital wide studies to confirm

Conclusion: This study provides one of the first reports of the detection of three cases of *mcr-1* plasmid mediated colistin resistance in Port Harcourt, Nigeria. The detection of such strains is worrisome considering the potential negative effect the spread of this gene has in the fight against drug resistance. More systematic studies would however need to be carried out to have a proper assessment of the spread and scope of the situation.

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