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
mediated by the mcr-1 gene which encodes an enzyme belonging to the phosphoethanolamine 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 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 report reported 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 5 min) 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’-GACCTCGTTTGTAGTTCCACAGA-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’-

GACCTCGTTTAGTTCCACAGA-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 gentamicin-antibiotics.

**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.

**Characteristics 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.
Prior to the detection of the \textit{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% to 14.3%. (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 2: Distribution of colistin resistance among isolates

<table>
<thead>
<tr>
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<th>Clinical (n = 13)</th>
<th>Non-Clinical (n = 22)</th>
<th>Total (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin resistant isolates</td>
<td>6 (46.2%)</td>
<td>15 (68.2%)</td>
<td>21 (60%)</td>
</tr>
</tbody>
</table>

Following the initial report of \textit{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 major 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 \textit{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 \textit{mcr-1} made these findings in non-clinical isolates rather than clinical isolates (Newton-Foot et al., 2017). The relatively low rate of 16.8% 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.

\textbf{Conclusion:} This study provides one of the first reports of the detection of three cases of \textit{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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