A novel report of colistin-resistant *Escherichia coli* carrying mcr-1 gene from animal and human feecal samples in Nigeria

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Abstract

**Background:** The mobilized colistin resistance (*mcr*)-1 gene confers transferable colistin resistance. Reports of *mcr*-1-positive *Escherichia coli* (MCRPE) have attracted substantial attention. However, in Nigeria, there is no report of *mcr*-1 gene resistance. Since colistin is a last resort for multiple drug-resistant isolates, this study therefore report the prevalence of *mcr*-1 gene among *E. coli* isolated from human and animal sources.

**Methods:** Out of a total of 280 samples collected from animal and human faecal samples from selected farms in Oyo and Osun States, Southwestern Nigeria between July 2015 and June 2016, 60 *E. coli* were identified using standard microbiological methods. The *mcr*-1 gene was detected in the isolates by conventional PCR assay.

**Results:** The *mcr*-1 gene was low and not statistically significant (*p*≤0.05). It was detected in 5 (8.3%) of 60 *E. coli* isolates (4= animals; 1= human)

**Conclusion:** This study is the first report of *mcr*-1 gene from *E. coli* from human and animal sources in Nigeria. This calls for urgent caution in the use of colistin in animal husbandry.

**Keywords:** Multidrug Resistance, *Escherichia coli*, food-producing animals, *mcr*-1, colistin resistance, Farms
INTRODUCTION

Colistin belongs to the last line of bactericidal antimicrobial drugs active against multidrug-resistant Gram-negative bacteria such as carbapenemase-producing Enterobacteriaceae. Consequently, the discovery of the plasmid-mediated colistin-resistant gene, mcr-1, in Escherichia coli in China [1] raises concern in the medical community because colistin might be useless in treating infections caused by mcr-1-carrying Enterobacteriaceae. For more than five decades in veterinary practices, gastrointestinal infections caused by Gram negative bacteria have been treated with colistin which is also used as a feed additive in food production to promote healthy development [2]. Colistin has been used in veterinary medicine through prophylactic or metaphylactic practices. The rising prevalence of multidrug-resistant (MDR) Gram-negative Enterobacteriaceae, especially carbapenem-resistant strains, necessitated a renewed interest in polymyxins, especially polymyxin E (colistin), for the management of Gram-negative infections in many countries [3,4]. Resistance to polymyxins has been traditionally regarded as occurring mutations in genes regulating the synthesis of LAra4N [5]. In Nigeria, there is no report of mobilized colistin resistance (mcr) 1 gene resistance and since colistin is a last resort for multiple drug resistance isolates, this study therefore aimed to determine the prevalence of mcr-1 gene among E. coli isolated from human and animal sources.

METHODOLOGY

Study area, sample collection and identification of E. coli
A total of 280 faecal samples recovered from animal and human were obtained in different farms and abattoirs in Osogbo, Nigeria were examined. A total of non-duplicate E. coli isolates (n=60) recovered from faecal samples collected from selected farms in Ogbomoso and Ibadan (Oyo State) and Osogbo and Ilesa (Osun State) South-western Nigeria between July 2015 and June 2016 were included in the study. The samples were collected as previously described [6,4] and confirmed using CHROMagar orientation Medium (Beckton Dickinson GmBH, Heidelberg, Germany). Isolates were stored at -80°C for further analysis. The farms were selected following farm owner’s voluntary consent to participate in the study.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing of the isolates was determined by the disk diffusion method according to the protocols recommended by CLSI [7]. using Amikacin, amoxycillin/clavulanic acid, ampicillin, Ampicillin/sulbactam, cephalaxin, cephalolin, ceftaxime, ceftazidine, cefoxitin, cefuroxime, chloramphenicol- C, ciprofloxacine- CIP, clindamycin- DA, doxycycline- DO, enrofloxacin, kanamycin, gentamicin, imipenem, levofloxacin, meropenem, neomycin, penicillin, Piperacillin/tazobactam, spectinomycin, streptomycin, Sulphamethoxazole, sulphamethoxazole/trimethoprim, tetracycline, ticarcillin, tobramycin and trimethoprim disks. The results were interpreted according to epidemiological cut-off (ECOFF) values recommended.

Minimum inhibitory concentration
The MIC of colistin for each isolate was determined by agar dilution test. Colistin MIC results were interpreted according to EUCAST criteria, in which E. coli strains with MIC of 2 µg/mL were considered colistin-resistant (COL-R).

Mcr-1 amplification and detection
DNA was extracted from each isolate using Quiagen extraction kit and mcr-1 PCR was carried out according to Chabou et al., [8] with slight modifications in primer base pair and PCR conditions (Table 1). Each PCR product was electrophoresed in 1.0% agarose gel with positive and negative controls included.

| Table 1. Primers and PCR conditions for amplification of mcr-1 gene |
|-------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Primer     | Sequence   | Denaturation | Annealing   | Extension | Cycles | Base pair |
| CLR5-F     | CCGTCA CTCCGTTTGTTC | 94°C-30s | 45°C-30s | 72°C-30s | 35 | 554 |
| CLR5-R     | CTTGTCAGTCTGA | GGG | | | | | |
Statistical Analysis

The prevalence of mcr-1 E. coli in the different categories was compared statistically with SAS (Statistical Analysis Software 92.2) using chi-square analysis. Prevalence was considered statistically significant at \( p \leq 0.05 \).

RESULTS

A total of the 60 isolates of E.coli were identified and all exhibited multidrug resistance (resistant to 3 or more antibiotics). Although all the isolates were 100% susceptible to carbapenems and varying degrees of resistance was observed with ampicillin, Ampicillin/sulbactam, cephalaxin, cephalosin, cefotaxime, ceftazidime, cefoxitin, cefuroxime, chloramphenicol, ciprofloxacin, doxycycline, enrofloxacin, levofloxacin, spectinomycin, streptomycin, Sulphamethoxazole, sulphamethoxazole/trimethoprim, tetracycline, ticarcillin and trimethoprim however exhibited varying degrees of the susceptibility to amikacin, cefoxitin, neomycin, piperacillin/tazobactam, kanamycin and other antibiotics. The prevalence of colistin resistance was low and not statistically significant (\( p \geq 0.05 \)). Colistin resistance was found in five isolates (8.3%): one human, 2 pig and 2 cattle. These five E. coli isolates were COL-R by all MIC methods with a MIC > 4 \( \mu \)g/ml. The five (8.3%) isolates carried mcr-1 gene out of which four were from animal and one from human sources. Table 2 showed characteristics of the isolates. Figure 1 showed the gel electrophoresis of mcr-1 amplicon in representative isolates.

Table 2: Sources and Quadruplex phylotyping of the five COL-R isolates

<table>
<thead>
<tr>
<th>Isolate no</th>
<th>Sources</th>
<th>Quadruplex phylotyping</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Human</td>
<td>E</td>
<td>1(1.7)</td>
</tr>
<tr>
<td>30 &amp; 31</td>
<td>Pig</td>
<td>E &amp; B2</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>40 &amp; 43</td>
<td>Cow</td>
<td>D &amp; B2</td>
<td>2 (3.3)</td>
</tr>
</tbody>
</table>

DISCUSSION

Despite the frequent use of colistin in animal farming for over 50 years, the occurrence of colistin resistance among E. coli strains isolated from food animals remains low (<1%) [9]. However, in this study, we found a prevalence of colistin resistance of 8.3% among commensal E. coli isolates from animals, especially pigs and cows. The frequency of resistance in commensal intestinal E. coli is considered to be a good marker for the selection pressure exerted by antibiotic use in the host animals and the resistance problems to be predicted in pathogenic bacteria [10]. The prevalence of colistin resistance in this study may be due to the increasing use of colistin in food animals in recent years. In veterinary medicine, COL is widely used, especially in pig and poultry production. The results obtained here is not unconnected to this act from farmers to boost production. It is doubtless that COL use in animals favours the selection and spread of resistance mechanisms. The mcr-1 positive E. coli from human source could probably be due to the unstable nature of the colistin resistance strains [3]. Despite this findings on human sample, it is still highly alarming as this may imply transfer of the gene from animal to food since many of the populace consume pig and cow meats in this environment as observed by previous authors [6,11]. Hence it is urgent to limit the usage of polymyxins (colistin) in veterinary medicine especially as feed additives in Nigeria.

Conclusion

The incidence of mcr 1 gene in commensal E. coli isolates in this study is alarming. This is the first report in Nigeria. Surveillance for the mcr-1 gene among clinically relevant Enterobacteriaceae species and other Gram-negative organisms infecting humans should be considered.

References


