The increase in the prevalence of infections caused by gram-negative pathogens that are multidrug resistant has prompted the reconsideration of polymyxins, colistin, and polymyxin B as valid therapeutic options. Isolates were susceptibility tested using reference broth microdilution. The description of a transformable polymyxin resistance gene in 2015 caused great concern. Enterobacteriaceae isolates carrying mcr genes encoding phosphoethanolamine-lipid A transferase that confers resistance to polymyxins have been reported globally among various species. The new variant, mcr-1, was detected for the first time in an E. coli isolate per patient was collected worldwide during 2016 as part of the SENTRY Antimicrobial Surveillance Program (SAP) and a new de novo mcr gene was characterized.

Materials and Methods

- **E. coli and K. pneumoniae clinical isolates were collected during 2016 from medical centers worldwide according to defined criteria, and 1 isolate per patient was collected.**
- **Isolates displaying colistin MIC ≤0.5 µg/mL (resistant by EUCAST criteria) were screened for mcr-1 genes by PCR and sequencing techniques.**
- **The novel variant and mcr-1 were cloned in pHET2.1 vector (Thermo Fisher Scientific, Waltham, Massachusetts, USA) transformed in an E. coli TOP10 host, and susceptibility tested.**
- **The mcr-1 naïve plasmid and other mcr-1-carrying isolates from the same medical center were characterized using next-generation sequencing and analysis (NGS) on MiSeq (Illumina, San Diego, California, USA).**

Sequences were de novo assembled and resistance determinants and plasmid incompatibility group encoding genes were searched using a curated library, applying criteria of >94% sequence identity and 40% minimum length coverage. Isolates displaying mcr-1 were identified in only 0.1% of the isolates tested, mostly in E. coli. The new variant, mcr-1, encoded similar activity against colistin when compared to mcr-1. This new variant is likely emerging via spontaneous mutation within an endemic plasmid structure. Although the prevalence of mcr-carrying isolates is low, the transferability of this colistin-resistance gene is concerning, and as such, a high diversity and widespread nature of this resistance determinant is critical. The recognition and understanding of this resistance mechanism will hasten the development of new treatment modalities and strategies.

**Table 1 Characteristics of mcr-1-producing isolates**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Polymyxin B MIC (µg/mL)</th>
<th>Polymyxin A MIC (µg/mL)</th>
<th>Other MIC values (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>≤0.12</td>
<td>4</td>
<td>≤0.03, ≤0.12, &gt;4</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>0.25</td>
<td>8</td>
<td>≤0.5, 0.12, &gt;4</td>
</tr>
</tbody>
</table>

**Table 2 Susceptibilities of transformants carrying mcr-1 and mcr-1T represented in the same background**

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
<th>mcr-1</th>
<th>mcr-1T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Cefsulodin</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Colistin</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Results**

- Among 11,493 E. coli and K. pneumoniae isolates, a total of 199 (1.7%) were resistant to colistin.
- None of the isolates tested were mcr-1 positive.
- Isolates carrying mcr-1 included 10 E. coli United States (2), Venezuela (2), Peru (2), Colombia (1), Poland (1), and 2 K. pneumoniae (1 each from Spain and Italy, Table 1).
- mcr-1-positive isolates were identified from bloodstream (5), urinary tract (3), and skin and skin structure (3) infections, or patients hospitalized with ≥3 days of healthcare facility stay.
- 8/12 isolates displayed MIC values ≤0.5 µg/mL, and the remaining isolates had colistin MIC values ≥8 µg/mL.
- Seven isolates were resistant to cephalosporins, but were susceptible to trimethoprim-sulfamethoxazole.
- 1/12 isolates were resistant to tetracycline and trimethoprim-sulfamethoxazole.

**Conclusion**

- Isolates carrying mcr-1 were identified in only 0.1% of the isolates tested, mostly in E. coli.
- The new variant, mcr-1T, encoded similar activity against colistin when compared to mcr-1.
- The mcr-1T variant likely emerged via spontaneous mutation within an endemic plasmid structure.
- Although the prevalence of mcr-carrying isolates is low, the transferability of this colistin-resistance gene is concerning, and as such, a high diversity and widespread nature of this resistance determinant is critical.