Prevalence of mcr-type genes among colistin-resistant Enterobacteriaceae collected in 2014-2016 as part of the INFORM global surveillance program

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Abstract

A set of 908 clinically derived colistin-resistant Enterobacteriaceae isolates collected worldwide in 2014–2016 were screened for the presence of the plasmid-borne mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 genes. In total 3.2% (29/908) of the collection were positive for mcr, including 27 Escherichia coli, 1 Klebsiella pneumoniae and 1 Enterobacter cloacae. Twenty-four isolates possessed genes from the mcr-1 family, including the original mcr-1 (n = 22), as well as mcr-1.2 (n = 1) and mcr-1.5 (n = 1), which each differ from mcr-1 by encoding single amino acid variations. Genes from the mcr-3 family were found in isolates from Thailand, including mcr-3.1 (n = 3) and mcr-3.2 (n = 1). An E. coli isolated from a patient with a urinary tract infection in Colombia contained the recently discovered mcr-5. The full colistin-resistant collection was tested against a panel of antimicrobial agents with ceftazidime-avibactam and tigecycline exhibiting the highest activity.

Introduction

Use of colistin, which became clinically available in 1959, has historically played a minor role as an anti-infective therapy due to its nephrotoxicity, as well as the availability of alternative antimicrobial agents [1]. However, the recent proliferation of multi-drug resistant (MDR) Gram-negative pathogens in the clinical setting threatens the efficacy of antibiotics across all classes. To bolster the number of so called “last resort” antimicrobial agents, polymyxins such as colistin are once again being administered clinically due to their potential effectiveness against MDR infections [2]. Until 2015, all characterized colistin resistance mechanisms were chromosomally encoded and thus only limited vertical transmission of resistance was envisioned [3]. However, the discovery by Liu, et al. [4] of the plasmid-borne phosphoethanolamine transferase resistance determinant mcr-1 revealed a mechanism for horizontal spread. MCR-1 and MCR-2, a protein with 80.7% identity to MCR-1 [5], have now been reported in Enterobacteriaceae worldwide [6–8]. In 2017, three additional MCR protein variants have been...
Pharmaceuticals, LP, which also included compensation fees for manuscript preparation. AstraZeneca’s rights to ceftazidime-avibactam were acquired by Pfizer in December 2016. G.S. was an employee of and shareholder in AstraZeneca Pharmaceuticals at the time of the study, and is currently an employee of Pfizer. M.W., M.E., D.S. and K.K. are employees of IHMA which received funding from AstraZeneca for the conduct of the study and development of this manuscript. None of the IHMA authors have any personal financial interest in the study sponsor (AstraZeneca Pharmaceuticals at the time of the study, and is currently an employee of and shareholder in AstraZeneca Pharmaceuticals). To disclose. This does not alter our adherence to all PLOS ONE policies on sharing data and materials.

Material and methods

The INFORM (International Network for Optimal Resistance Monitoring) global surveillance program monitors antimicrobial resistance to a variety of pathogens isolated from intra-abdominal, urinary tract, skin/soft tissue, lower respiratory tract and, as of 2014, blood infections [12]. During 2014–2016, the program received a total of 44,407 isolates of Enterobacteriaceae including those collected by 87 medical center laboratories located in 18 countries in Europe (n = 21,461), 36 medical center laboratories in 9 countries in the Asia/Pacific region (n = 7,215), 24 medical center laboratories in 6 countries in Latin America (n = 7,180), 17 medical center laboratories in 5 countries in the Middle East/Africa region (n = 3,707) and 26 medical center laboratories in the United States (n = 4,844). All isolate species identifications were confirmed in the central laboratory by MALDI-TOF MS (Bruker Daltonics, Waltham, Massachusetts). Not including Serratia spp. and members of the tribe Proteaceae (genera Proteus, Providencia and Morganella), which are intrinsically colistin non-susceptible, 934 isolates were found to be resistant to colistin by broth microdilution [13] at an MIC ≥ 4 μg/mL, which is the EUCAST resistance breakpoint for the Enterobacteriaceae [14]. Of these, 908 isolates were available to screen, as no isolates could be obtained from China in 2014–2016 or Hong Kong in 2015–2016 due to export restrictions. The species composition of the complete set included Citrobacter freundii (n = 6), Citrobacter koseri (n = 3), Enterobacter aerogenes (n = 18), Enterobacter asburiae (n = 143), Enterobacter cancerogenus (n = 1), Enterobacter cloacae (n = 165), Enterobacter kobei (n = 11), Escherichia coli (n = 64), Hafnia alvei (n = 1), Klebsiella oxytoca (n = 13), Klebsiella pneumoniae (n = 481) and Klebsiella variicola (n = 2).

The collection was investigated for the presence of the colistin-resistance conferring mcr genes by several PCRs. The initial reaction utilized a custom primer set designed to amplify a 143 bp region common to both mcr-1 and mcr-2 (MCR-Univ-F: 5’-CTGTGCGGTGATGTTT CAGC-3’ and MCR-Univ-R: 5’-CACGCCTTTTGAGCTGAAT-3’). Primers that anneal to 16S rRNA gene (U341F, 5’-CCTACGGGRSGACGAG-3’; U519R 5’-GWATTACCGCGGC KTGG-3’) were included in the reaction as an internal positive control for amplification. Subsequently, a multiplex PCR was employed with primers MCR3-F and MCR3-R [9], and MCR-4 FW and MCR-4 RV [10] to detect the mcr-3 and mcr-4 genes, respectively. This reaction also included the 16S rDNA internal positive control. Finally, the screening for mcr-5 utilized MCR5-intern_fw and MCR5-intern_rev primers [11], along with the internal 16SrDNA control. As external positive controls, synthetic DNA constructs were employed for each of the mcr genes (IDT Inc., Coralville, Iowa). All screen-positive results were confirmed by PCR amplification using custom-designed primers flanking the coding region and sequencing the gene in full (mcr-1, exgenMCR1-F, 5’-CCGAAATTATCCACCGGT-3’ and exgenMCR1-R, 5’-CCCATGACAGGCCGATAC-3’; mcr-3, exgenMCR3-F, 5’-TCGTTAGAAAGTGATTGT TGGAC-3’ and exgenMCR3-R, 5’-CCTCTTCTGATTTGCCGCTG-3’; mcr-5, exgenMCR5 F, 5’-AACCGTTGAAGGACGAGA-3’ and exgenMCR5-R, 5’-CCATGAGCCTCGTG ATCCCC-3’). Sequence variants were assigned based upon comparison to sequences deposited in the NCBI databases. mcr-positive E. coli underwent multilocus sequence typing based on the partial sequences of adk, fumC, gyrB, icd, mdh, purA, and recA (https://enterobase. warwick.ac.uk/species/index/ecoli).
Results and discussion

In total, *mcr* was detected in 29 isolates (3.2%), and included 27 *E. coli*, 1 *K. pneumoniae* and 1 *E. cloacae* collected in 15 countries (Malaysia, 5; Thailand, 5; Spain, 3; Argentina, 2; Italy, 2; Colombia, 2; Germany, 2; Brazil, Hong Kong, Poland, Portugal, Russia, South Africa, Taiwan, and Venezuela, 1 each) as part of INFORM in 2014 (n = 14), 2015 (n = 11) and 2016 (n = 4) (Table 1). Twenty-two isolates harbored the original *mcr*-1 gene, one isolate carried the gene for the single amino acid variant (Q3L) MCR-1.2 [15], and one isolate carried *mcr*-1.5, that codes for another single amino acid variant, (H452Y). Four *E. coli* isolates, all originating from Thailand, were found to possess *mcr*-3, with three harboring the original *mcr*-3.1 [9] and one possessing the gene coding for the single amino acid variant, MCR-3.2 (T488I). An *E. coli* strain from Colombia was shown to carry the recently discovered *mcr*-5 gene [11]. No *mcr*-2 or *mcr*-4 genes were identified.

As part of the INFORM surveillance program, organisms non-susceptible to meropenem, resistant to ceftazidime, and/or positive for ESBL activity qualify for β-lactamase gene screening. Thirteen of the 29 *mcr* positive isolates qualified and were screened for genes encoding acquired ESBLs, AmpC β-lactamases, serine carbapenemases (*bla*KPC, *bla*OXA-48, *bla*GES), and metallo-β-lactamases by PCR and DNA sequencing, as previously described [16]. Nine *mcr*-positive isolates were found to carry CTX-M-type ESBLs either alone or in combination with AmpC-type β-lactamases and/or original-spectrum β-lactamases (OSBL) of the TEM or SHV type. Four possessed a CMY-2 AmpC-type enzyme either alone or with a TEM-OSBL, and in one case with a CTX-M-161 enzyme. None of the *mcr*-positive isolates carried carbapenemases. Of note, each of the four *mcr*-3 gene family-harboring isolates also carried the CTX-M-55 ESBL variant, known to be common in Asia especially in *E. coli* isolated from veterinary sources [17].

All *mcr* containing isolates were susceptible to meropenem (MIC < 2 μg/mL) and doripenem (MIC < 2 μg/mL), and 62.1% (18/29) were susceptible to both ceftazidime (MIC < 8 μg/mL) and aztreonam (MIC < 8 μg/mL) by CLSI breakpoints [18]. However, the addition of 4 μg/mL avibactam rendered 100% of the isolates susceptible (MIC < 8 μg/mL) to ceftazidime (using FDA recommended breakpoints [19]). All isolates harboring *mcr* were also susceptible (MIC ≤ 2 μg/mL) to tigecycline (using FDA recommended breakpoints [20]). The *in vitro* activity of several antimicrobials against the full set of 908 colistin-resistant isolates is given in Table 2. Ceftazidime-avibactam, along with tigecycline, were the most active agents against these isolates. The addition of avibactam to ceftazidime rendered 97.5% of the population susceptible (FDA breakpoints [19]), as compared to just 43.8% susceptibility with ceftazidime alone (CLSI breakpoints [18]).

The *mcr*-positive *E. coli* were distributed among several lineages, with the ST10 clonal complex (including ST167, ST744 and ST48) the most abundant (n = 6). *mcr*-harboring *E. coli* from this group has been reported on numerous occasions, for example ST10 from human clinical samples in China [21], ST744 from human and cattle-associated samples in Europe [22, 23], ST167 from human infections in Spain and China [24, 25], as well as ST48 from hospital sewage and human clinical samples, in China and Switzerland, respectively [26, 27]. Additional worldwide clones previously shown to harbor *mcr* were also confirmed here, and include ST641 [28], ST410 [29,30], and ST156 [31, 32]. Our screening identified two *mcr*-harboring ST117 *E. coli* (and a ST117 single-locus variant with a novel *fumC*), one of which carried the MCR-3.2 gene. ST117 is a clonal group associated with poultry disease [33] and *mcr*-type genes have only rarely been observed in this clone [27, 34]. Of particular interest, one isolate from Brazil typed as a single locus variant (novel *purA*) of the pathogenic *E. coli* ST131 [35]. ST131 often exhibits an extended spectrum β-lactamase (ESBL) phenotype and frequently possess CTX-M-15; however, this Brazilian isolate was susceptible to third-generation cephalosporins. In general, the fact that *mcr*-type genes have been found in *E. coli* of such diverse STs
from food, human and animal specimens suggests the spread of these genes is linked more to successful plasmids and mobile elements rather than single specific E. coli clones [27].

Overall, the prevalence of mcr observed here is in accordance with previous reports from large global surveillance studies. For example, Castanheira, et al. noted that 4.9% (19/390) of a

Table 1. mcr positive Enterobacteriaceae collected as part of the INFORM global surveillance program during 2014–2016.

| Year | Country | Organism          | Clinical Sample | MIC (µg/mL)* | MLST | mcr gene product | β-Lactamase content |
|------|---------|-------------------|-----------------|--------------|------|-----------------|---------------------|
| 2014 | Colombia| Escherichia coli  | Urine           | 4 0.25 32 0.06 0.25 CST CAZ AVI CAZ MEM TGC | ST641 | MCR-5            | CMY-2               |
| 2014 | Germany | Escherichia coli  | GI tract: appendix | >4 0.06 0.25 0.03 0.12 MCR-1 | ST46  |                 | NC                 |
| 2014 | Hong Kong| Escherichia coli | Blood           | 4 0.06 0.12 0.03 0.25 MCR-1 | ST10  |                 | NC                 |
| 2014 | Italy   | Escherichia coli  | Wound           | 4 0.12 0.25 0.015 0.25 MCR-1 | ST744 |                 | NC                 |
| 2014 | Italy   | Escherichia coli  | Blood           | 4 0.12 0.25 0.015 0.25 MCR-1 | ST453 | MCR-1.2          | NC                 |
| 2014 | Malaysia| Escherichia coli  | Abscess         | 4 0.12 16 0.03 1 MCR-1 | ST10  |                 | TEM-OSBL; CTX-M-15  |
| 2014 | Malaysia| Escherichia coli  | Gangrene        | 4 0.03 16 0.03 0.5 MCR-1 | ST162 |                 | TEM-OSBL; CMY-2     |
| 2014 | Portugal| Enterobacter cloacae | Wound >4 0.25 1 0.06 1 MCR-1 | ST1167 | MCR-1 |                 | TEM-OSBL; CTX-M-1   |
| 2014 | Russia  | Escherichia coli  | Peritoneal fluid | >4 0.12 2 0.03 0.25 MCR-1 | ST156 |                 | TEM-OSBL; CTX-M-1   |
| 2014 | South Africa| Escherichia coli | Wound           | 4 0.03 0.5 0.03 0.25 MCR-1 | ST602 |                 | NC                 |
| 2014 | Spain   | Escherichia coli  | Peritoneal fluid | >4 0.12 0.25 0.015 0.5 MCR-1 | ST117 |                 | NC                 |
| 2014 | Spain   | Escherichia coli  | Blood           | 4 1 64 0.12 2 MCR-1 | ST167 |                 | TEM-OSBL            |
| 2014 | Taiwan  | Escherichia coli  | Wound           | 4 0.25 32 0.06 0.25 MCR-1 | ST117 | MCR-1            | TEM-OSBL; CTX-M-161; CMY-2 |
| 2014 | Thailand| Klebsiella pneumoniae | Wound | 4 0.5 64 0.06 0.5 MCR-1 | ST602 |                 | NC                 |
| 2015 | Argentina| Escherichia coli | Urine           | 4 0.12 0.5 0.03 0.25 MCR-1 | ST48  | MCR-1.5          | NC                 |
| 2015 | Argentina| Escherichia coli | Peritoneal fluid | 8 0.25 8 0.06 0.5 Novel | ST117 | MCR-1            | NC                 |
| 2015 | Colombia| Escherichia coli  | Wound           | 4 0.12 0.25 0.03 0.5 MCR-1 | ST744 |                 | NC                 |
| 2015 | Malaysia| Escherichia coli  | Blood           | 4 0.03 0.25 0.03 0.5 MCR-1 | ST2705|                 | NC                 |
| 2015 | Malaysia| Escherichia coli  | Wound           | 4 0.12 4 0.03 0.25 MCR-1 | ST5907|                 | TEM-OSBL; CTX-M-65  |
| 2015 | Malaysia| Escherichia coli  | Peritoneal fluid | 4 0.06 0.12 0.03 0.12 MCR-1 | ST97 |                 | NC                 |
| 2015 | Spain   | Escherichia coli  | Endotracheal aspirate | 4 0.12 0.25 0.03 1 MCR-1 | ST88  |                 | NC                 |
| 2015 | Thailand| Escherichia coli  | Wound           | 4 0.5 >128 0.12 2 MCR-1 | ST1193|                 | NC                 |
| 2015 | Thailand| Escherichia coli  | Blood           | 4 0.12 8 0.03 0.25 MCR-1 | ST117 | MCR-3.2          | TEM-OSBL; CTX-M-55  |
| 2015 | Thailand| Escherichia coli  | Abscess         | 4 0.12 16 0.06 0.25 MCR-1 | ST410 | MCR-3.1          | CTX-M-55            |
| 2015 | Venezuela| Escherichia coli | Abscess         | 4 0.12 0.25 0.03 0.5 MCR-1 | ST97 |                 | NC                 |
| 2016 | Brazil  | Escherichia coli  | Peritoneal fluid | 4 0.12 0.25 0.03 0.25 Novel | ST117 | MCR-1            | NC                 |
| 2016 | Germany | Escherichia coli  | Wound           | 4 0.12 0.25 0.03 0.25 MCR-1 | ST175 | MCR-1            | NC                 |
| 2016 | Poland  | Escherichia coli  | Wound           | 4 0.12 0.25 0.06 0.25 MCR-1 | ST12  |                 | NC                 |
| 2016 | Thailand| Escherichia coli  | Blood           | 4 0.12 16 0.12 0.12 MCR-1 | ST4546| MCR-3.1          | TEM-OSBL; CTX-M-55  |

*MICs performed via broth microdilution (13); CST, colistin; CAZ, ceftazidime; CAZ-AVI, ceftazidime with 4 µg/mL avibactam; MEM, meropenem; TGC, tigecycline.

*As part of INFORM, meropenem non-susceptible, ceftazidime-resistant, and phenotypically positive ESBL isolates were screened for genes encoding acquired extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, serine carbapenemases (KPC, OXA-48, GES), and metallo-β-lactamases (MBL) by PCR and DNA sequencing as previously described (16).

*NC = not characterized

*OSBL = original spectrum β-lactamase (eg. TEM-1, SHV-1, SHV-11)

*Single-locus variant (novel fumC) of E. coli ST117

*Single-locus variant (novel purA) of pathogenic E. coli ST131

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Table 2. In vitro activity of selected antimicrobials against 908 colistin-resistant Enterobacteriaceae collected worldwide during 2014–2016.

| Drug          | MIC Interpretive criteria (S/I/R) | % Susceptible | % Intermediate | % Resistant | MIC_50 μg/mL | MIC_90 μg/mL | MIC Range μg/mL |
|---------------|----------------------------------|---------------|----------------|-------------|--------------|--------------|-----------------|
| Amikacin      | ≤16/32/≥64                       | 78.6          | 11.3           | 10.1        | 2            | >32          | 0.5 - >32       |
| Ceftazidime   | ≤4/8/4/≥16                       | 43.9          | 2.0            | 54.1        | 32           | >128         | ≤0.015 - >128   |
| Ceftazidime-avibactam | ≤8 /na/≥16    | 97.7          | na             | 2.3         | 0.25         | 2            | ≤0.015 - >128   |
| Colistin      | ≤2 /na/≥4                        | 0             | na             | 100.0       | 8            | >8           | 4 - >8         |
| Levofloxacin  | ≤2/4/≥8                          | 52.6          | 2.9            | 44.5        | 2            | >8           | 0.015 - >8     |
| Meropenem     | ≤2/4/≥8                          | 70.4          | 3.2            | 26.5        | 0.12         | >8           | 0.008 - >8     |
| Tigecycline   | ≤2/4/≥8                          | 95.6          | 4.0            | 0.4         | 0.5          | 2            | 0.03–8         |

^MICs were interpreted according to CLSI breakpoints [18], with the exception of ceftazidime-avibactam, for which MICs were interpreted using criteria according to the FDA [19], colistin for which EUCAST breakpoints were utilized [14] and tigecycline, for which MICs were interpreted using FDA criteria [20]; S, susceptible; I, intermediate; R, resistant; na, not applicable (no intermediate breakpoint).

^Avibactam concentration fixed at 4 μg/mL.

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worldwide colistin-resistant collection of E. coli and K. pneumoniae from the SENTRY program contained mcr-1, and 32.3% (19/59) of the resistant E. coli contained this gene [36]. mcr was also enriched in the colistin-resistant E. coli population examined here, as 42.2% (27/64) of the resistant isolates from this species harbored mcr with the remainder presumably possessing a chromosomally-encoded resistance determinant. It should be noted that mcr has been discovered in isolates susceptible to colistin [37], so the actual frequency of occurrence could be higher. In this study, mcr-1 was observed exclusively in E. coli except for an E. cloacae isolate originating from Portugal. Until recently, mcr-1 positive E. cloacae were only reported from Asia [38, 39]; however, the geographic range was expanded with the discovery of a clinical E. cloacae isolate with mcr-1 in France [40]. The mcr-5 harboring E. coli and K. pneumoniae from Thailand confirm the previous report of the presence of this gene in clinical isolates from this country [9]. Finally, finding mcr-5 in a Colombian E. coli clinical isolate expands both its geographic and host range, as at the time of this writing mcr-5 has only been confirmed in Salmonella enterica Paratyphi B isolated from food animals and food products in Germany, and in E. coli from porcine clinical specimens in Japan [41]. This gene was found in silico to be present the genome of a Cupriavidus gilardii from the U.S., and mcr-5 has been reported to be located on a unique Tn3-type transposon in both S. enterica Paratyphi B and C. gilardii [11]. Although we did not sequence this complete region, the forward mcr-5 flanking primer utilized to amplify the full coding region overlaps the 3’ end of the chromate reductase gene, chrB, directly upstream of mcr-5 in the Tn3-type transposon, and the reverse flanking primer anneals to the 5’ portion of the MFS-type transporter gene, immediately downstream of mcr-5 in the transposon arrangement [11], suggesting a similar genetic orientation in this Colombian strain.

In summary, this report confirms the global spread of mcr. Notably we did not find the coexistence of mcr with any carbapenemase genes, although co-carriage is being increasingly reported, including mcr-1 with blaNDM in Enterobacteriaceae from the U.S. and China [32, 42–46], as well as mcr-1 and blaKPC in isolates from Singapore [47]. Continual surveillance of this recently recognized threat to public health is warranted as MDR bacteria that acquire mcr will leave few treatment options.

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