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Short Communication

Prevalence and antibiotic susceptibility of colistin-resistance gene (*mcr-1*) positive *Enterobacteriaceae* in stool specimens of patients attending a tertiary care hospital in Singapore

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ABSTRACT

Objectives: The aim of this study was to determine the prevalence of the colistin-resistance gene (*mcr-1*) and the antibiotic-susceptibility profile of *mcr-1* positive, colistin-resistant isolates in stool specimens of patients attending a tertiary care hospital in Singapore.
Methods: 201 diarrheal stool specimens of patients attending the Changi General Hospital between May to August 2017 were collected and screened for the presence of *mcr-1* by culture and molecular methods. Antibiotic-susceptibility profile of *mcr*-1 positive isolates was determined using the polymyxin B and colistin E-tests and the VITEK 2 system.
Results: We observed an unexpectedly high prevalence of *mcr-1* in patients attending a tertiary care hospital in Singapore, i.e 6.0% and 8.0% estimated by stool culture and direct stool PCR, respectively. The *mcr-1* gene was detected predominantly in *Escherichia coli*. Antibiotic-susceptibility testing on 12 *mcr-1* positive *Enterobacteriaceae* isolates revealed variable susceptibility profiles with no detection of carbapenem-resistant *Enterobacteriaceae*.
Conclusions: This is the first report of the prevalence of human faecal carriage of *mcr*-1 in Singapore. Our findings highlight the potential risk of *mcr*-1 spread among our patient cohort. The *mcr-1* gene detection combined with the detection of other resistance gene targets of clinical importance is recommended to pre-empt the spread *mcr-1* in our patients.

Colistin is one of the few remaining therapeutic options available to treat infections due to carbapenem-resistant *Enterobacteriaceae*. The recently recognised global distribution of a transferable, plasmid-borne colistin resistance determinant (*mcr-1* gene) has raised concern because plasmid borne colistin-resistance can be transmitted between bacteria more easily than colistin resistance resulting from chromosomal mutation (Du et al., 2016). In Singapore, the positive rate of *mcr-1* among clinical *Enterobacteriaceae* isolates was previously estimated (Teo et al., 2016) but local data on the prevalence of human faecal carriage of *mcr*-1 has not been documented. We therefore set out to investigate the prevalence of the *mcr-1* gene in stool specimens of patients attending a tertiary care hospital and to determine the antibiotic-susceptibility profile of *mcr-1* positive *Enterobacteriaceae* strains isolated from those patients.

A prospective study was conducted on 201 diarrheal stool specimens submitted for stool culture or *Clostridium difficile* PCR from adult patients attending Changi General Hospital between May to August 2017. Changi General Hospital locates in eastern Singapore and the patient population attending here are mainly Singaporeans and Singapore permanent residents living in the east and north-east regions of the island. Stool specimens were pre-treated with ASL-buffer and InhibitEx tablets (Qiagen, Hilden, Germany) prior to nucleic acid extraction with EZ1 Virus Mini kit v2.0 (Qiagen). A laboratory developed real-time PCR was performed using primers and probes mcr-1_F (5′-ATGGCACGGTC-TATGATA-3′), mcr-1_R (5′-CGGATAATCCACCTTAACA-3′), mcr-1_P (FAM-BHQ 5′-CTACAGACCGACCAAGCCGA-3′) for *mcr-1* detection

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(Nijhuis et al., 2016); and MS2_F (5'-TTAAATCGGCT ACGGGGTTG-3'), MS2_R (5'-GAGGAGAGCCGTACCCACAC-3'), and MS2_P (TYE705-IAbRQSp 5'-CGGAGGTATGTCAGATCCACGC-3') for internal control detection. The real-time PCR assay was performed using Maxima probe qPCR Master Mix (Thermo Fisher Scientific, Pittsburgh, USA) on Rotor-Gene Q 5plex system (Qiagen) as reported previously (Nijhuis et al., 2016). For any sample in which both internal control and *mcr-1* were not detected in the original nucleic acid extract, the PCR was repeated on the 1:10 dilution of the nucleic acid extract. The assessed assay parameters showed linearity $R^2 = 0.998$ and amplification efficiency of 93.4%, with estimated 95% limit of detection of 15 cfu/reaction using Probit regression analysis.

Stool specimens were concurrently screened for colistin resistant enteric bacteria using SuperPolymyxin selective medium (Nordmann et al., 2016). All suspected bacteria colonies isolated on the selective medium were screened for the presence *mcr-1* using the real-time PCR assay as described above and subjected to bacterial identification using the VITEK MS system (BioMérieux, Marcy l'Etoile, France). All the *Enterobacteriaceae* isolates picked up by culture were also tested for polymyxin B and colistin susceptibility by Etest (BioMérieux). Antibiotic susceptibility testing of *mcr*-1-positive isolates was performed with the Vitek AST-N257 card (BioMérieux) for all other antimicrobials.

Out of 23 *Enterobacteriaceae* isolates from screening culture (excluding all *Proteus spp., Morganella spp., Providentia spp., Serratia spp.)*, 17 *Enterobacteriaceae* isolates displayed colistin MICs or polymyxin B MICs (COL/PB MICs) of >2 mg/L, which is the resistant breakpoint for colistin according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. If the resistant breakpoint was lowered to >1 mg/L to improve detection of *mcr-1* positive bacteria according to the study published by Chew and coworkers (Chew et al., 2017), 19 *Enterobacteriaceae* isolates displayed COL/PB MICs of >1 mg/L. However, the *mcr-1* gene was only detected in 12 isolates, i.e 1 *Klebsiella pneumoniae* and 11 *Escherichia coli* isolates. The *mcr-1* prevalence determined by screening culture was estimated to be 6.0%. The polymyxin B MICs and colistin MICs of those *mcr-1* positive isolates ranged from 3 mg/L to 12 mg/L, and from 3 mg/L to 6 mg/L, respectively. For the *mcr*-1 negative isolates with COL/PB MICs of >1 mg/L, the *mcr-2* conventional PCR was performed but no *mcr-2* was found (unpublished data).

Antibioltic-susceptibility testing on 12 *mcr-1* positive *Enterobacteriaceae* isolates revealed variable susceptibility profiles (Figure 1). Extended-spectrum beta-lactamase (ESBL) was detected in an *E. coli* isolate and AmpC detected in a *K. pneumoniae* isolate using Kirby Bauer disc diffusion screening method -based on Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines (Table 1).

On the other hand, direct PCR of stool specimens detected a higher *mcr-1* rate of 8.0% although PCR inhibition was present in 3.0% of specimens. Overall the *mcr-1* gene was detected by both culture and direct PCR in 10 specimens, by direct PCR only in 6 specimens and by culture only in 2 specimens. There was no *mcr-1* positive bacteria isolated from the samples having PCR inhibitors. Compared to culture method on SuperPolymyxin selective medium, the sensitivity, specificity, positive and negative predictive values of direct PCR are 83.33%, 96.72%, 62.55% and 98.88%, respectively.

Overall, the *mcr-1* prevalence determined by both culture and PCR methods was estimated to be 9.0% (95% confidence interval of 5.7%–13.7%, Wilson score interval). The *mcr-1* prevalence in our patient cohort was considerably higher than the 0.98% *mcr-1* prevalence of clinical *Enterobacteriaceae* isolates cultured from blood, urine and miscellaneuous samples in a previous study in Singapore (Teo et al., 2016). As the sample size of our study was relatively small and the specimen type was solely diarrheal stool, the percentage attributed to true community-associated cases remains unclear. Compared with human faecal carriage data of other regions, the *mcr-1* positive rate reported in our study was higher than that observed in Guangzhou (6.2%) (Zhong et al., 2018), Shangdong (4.9%) (Bi et al., 2017), and Hongkong (2.08%) (Chan et al., 2018), but lower than the rates reported in four different hospitals located in Shenzhen city and adjacent areas in China (28%) (Chen et al., 2017). Further study with larger cohort from different representative populations, geographical locations and demographic charateristics is necessary to provide a more holistic picture on the prevalence of *mcr-1* in our local context.

Interestingly, similar to observation in several other studies (Chen et al., 2017; Teo et al., 2016; Wise et al., 2018), all 12 *mcr-1*-positive isolates were sensitive to carbapenems. Nevertheless, the potential risk of *mcr-1* spread to carbapenem-resistant *Enterobacteriaceae* within hospitals and community should not be undermined.

The high *mcr-1* prevalence found in this study highlights the importance of monitoring the potential risk of *mcr*-1 spread among our patient cohort. Continued surveillance of multidrug-resistance in Gram-negative enteric bacteria including detection of ESBL genes and carbapenemase genes combined with *mcr-1* gene detection can pre-empt the spread of *mcr-1* in our patients.

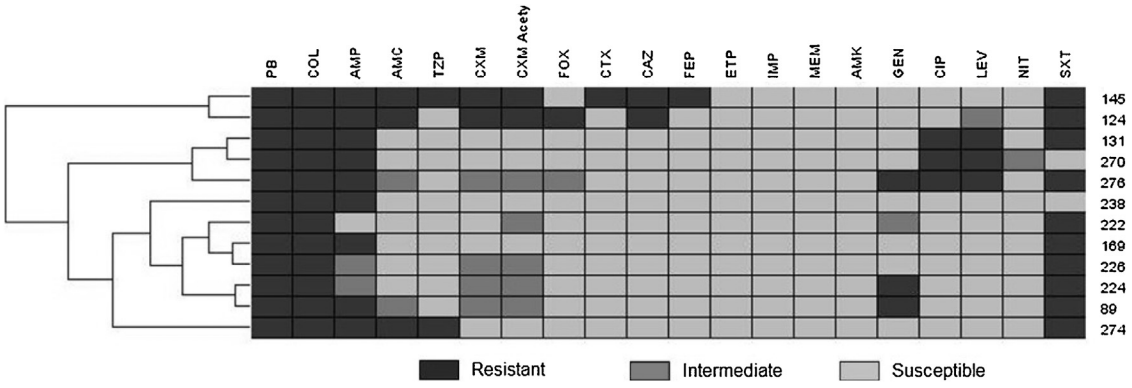

Figure 1. Hierarchical clustering, which was constructed using complete linkage algorithm in GENESIS v1.8.1 software (Sturn et al., 2002), exhibited variable antimicrobial susceptibility profiles of *mcr-1*-positive *Enterobacteriaceae* isolates. Antibiotics colored in dark, medium, and light gray correspond to those with resistant, intermediate, or susceptible interpretation results, respectively. For colistin, susceptible breakpoint of ≤2 mg/L and resistant breakpoint of >2 mg/L was applied according to the EUCAST guidelines. For polymyxin B, colistin breakpoints were applied. The interpretation of susceptibility testing was based on the CLSI guidelines for other antibiotics.

Table 1
Features of *mcr-1*-positive ESBL-producing *E. coli* isolate and AmpC-producing *K. pneumoniae* isolate.

Isolate	Species	Date of isolation	Resistance phenotype	MIC (mg/L)[a]														
				PB	COL	IMP	MEM	ETP	CXM	FOX	CTX	CAZ	FEP	AMP	AMC	TZP	AMK	GEN	CIP	LEV
Isolate 124	*E. coli*	20/6/2017	ESBL	3	3	≤0.25	≤0.25	≤0.5	32	≥64	2	16	≤1	≥32	≥32	8	4	≤1	1	4
Isolate 145	*K. pneumoniae*	29/6/2017	AmpC	4	3	≤0.25	≤0.25	≤0.5	≥64	≤4	≥64	≥64	≥64	≥32	≥32	≥128	≤2	≤1	1	1

[a] Abbreviations: PB-polymyxin B, COL-colistin, IMP-imipenem, MEM-meropenem, ETP-ertapenem, CXM-cefuroxime,FOX-cefoxitin, CTX-cefotaxime, CAZ-ceftazidime, FEP-cefepime, AMP-ampicillin, AMC-amoxicillin/clavuclanic acid, TZP-piperacillin/tazobactam, AMK-amikacin, GEN-gentamicin, CIP-ciprofloxacin, LEV-levofloxacin, NIT-nitrofurantoin, SXT-co-trimoxazole.

Conflict of interest

All authors have no conflict of interests to declare.

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