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Colistin-Resistant *mcr-1*-Positive *Escherichia coli* on Public Beaches, an Infectious Threat Emerging in Recreational Waters

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ABSTRACT The emergence and rapid spread of colistin-resistant *Escherichia coli* carrying the *mcr-1* gene have generated an urgent need to strengthen surveillance. We performed a meticulous investigation of strains of this sort, which resulted in the identification of international clones of *E. coli* carrying IncX4-plasmid-mediated *mcr-1* and bla_{CTX-M} genes in recreational waters of public urban beaches in cities with high tourist turnover, highlighting a new environmental reservoir.

KEYWORDS MCR-1, ESBL, CTX-M, IncX4, polymyxins, Brazil

The emergence and rapid spread of colistin-resistant *Enterobacteriaceae* carrying the *mcr-1* gene have generated a profound sense of public alarm (1). *Escherichia coli*, one of the bacterial species that is most widely distributed and exchanged between the environment, animals, and humans, has been the main host of *mcr-1* (2, 3). In South America, the occurrence of *E. coli* carrying *mcr-1* and bla_{CTX-M} genes in human (4–6) and wild animal (7) infections and food-producing animals (8) has created an urgent need to strengthen epidemiological surveillance. Using a whole-genome sequencing (WGS) approach, we performed a meticulous investigation of strains of this sort, which resulted in the identification of international clones of *E. coli* carrying *mcr-1* and bla_{CTX-M-}type genes in recreational waters of public urban beaches and highlighted a new source of transmission of this infectious threat.

In September 2016, coastal water samples were collected from 11 different public beaches (in the southeastern Brazilian continental margin of São Paulo State) surrounding urban counties with a population of about 800,000 inhabitants, which can double during the summer. Following standard methods for the examination of water and wastewater (http://www.standardmethods.org), 500-ml surface water samples were collected, on the same day, in sterile bottles, transported to the laboratory in cooled containers (at about 4°C to 10°C), and processed within 6 h. From each water sample, 100 ml was concentrated by filtration through sterile membrane filters with a pore size of 0.45 μm. The filters were placed on MacConkey agar plates and incubated for 24 h at 37°C. Next, the membrane filters were aseptically removed and placed separately in sterile tubes that had been filled previously with 10 ml of sterile Mueller-Hinton broth. After vortex mixing, an aliquot (100 μl) of each culture was streaked on MacConkey agar plates supplemented with colistin (2 μg/ml).

Three colistin-resistant *E. coli* strains were recovered from different beaches located in the cities of São Vicente and Santos (Fig. 1); the latter is the major beachfront city of the region, with the largest shipping terminal in Latin America. The isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrom-

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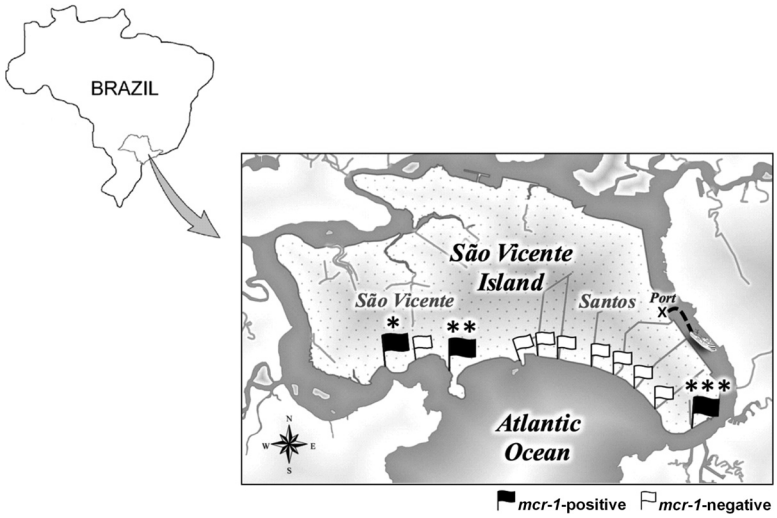

FIG 1 Map showing sampling locations (represented by flags) on public beaches surrounding the area of Santos and São Vicente cities, in the southeastern Brazilian continental margin of São Paulo State. MCR-1-positive *E. coli* strains (black flags) were isolated from seawater at recreational beaches in São Vicente (*, ICBEC2AM [location, −23.974697S, −46.395060W]; **, ICBEC3AM [location, −23.974995S, −46.371613W]) and Santos (***, ICBEC13AM [location, −23.986450S, −46.309086W]).

etry (MALDI-TOF MS) analysis, and antimicrobial susceptibility profiles and polymyxin MICs were determined by using the disc diffusion and broth microdilution methods, respectively (9, 10). Additionally, imipenem and meropenem MICs were determined by using the Etest method, and all isolates displayed susceptibility to imipenem (MICs of ≤0.19 μg/ml) and meropenem (MICs of ≤0.032 μg/ml).

DNA libraries from ICBEC2AM and ICBEC3AM *E. coli* isolates were sequenced using the NextSeq platform with paired-end reads (Illumina), whereas the DNA library from ICBEC13AM *E. coli* was sequenced using the MiSeq platform with paired-end reads (Illumina). Serotypes (STs), multilocus sequence typing (MLST), plasmid replicons, antimicrobial resistance genes, and *E. coli* virulence genes were identified or performed using multiple databases, i.e., SerotypeFinder 1.1, MLST 1.8, PlasmidFinder 1.3, ResFinder 2.1, and VirulenceFinder 1.5, respectively, available from the Center for Genomic Epidemiology.

The presence of *mcr-1* and other clinically important resistance genes, including the extended-spectrum β-lactamase (ESBL) genes bla_{CTX-8} and $bla_{CTX-M-1}$, conferred a multidrug resistance (MDR) phenotype to *E. coli* strains belonging to the globally reported sequence types ST10, ST46, and ST1638 (Table 1). ST10 and ST46 encompass pathogenic strains responsible for human and animal infections, as reported for *E. coli* (7, 11, 12). Interestingly, the isolation of an *E. coli* ST10 strain carrying the *mcr-1* gene from a water sample collected from a public beach on the coast of Santos city and the isolation of an *E. coli* ST10 strain from an infected migratory Magellanic penguin suffering from pododermatitis, in the same area, in an earlier study by our group (7) suggest that the ubiquitous ST10 survives easily and also spreads in the marine environment. Indeed, all *E. coli* stains identified in this study showed tolerance to NaCl concentrations up to 10% (Table 1). Recent studies have reported observation of the coexistence of *mcr-1* and bla_{CTX-M} in MDR *E. coli* strains belonging to the ST10 complex in well water in rural China (13), identification of environmental *mcr-1*-positive *E. coli* isolates surrounding German swine farm areas (14), and isolation of *mcr-1*-positive *E. coli* strains from diseased food-producing animals in China (15) and in France and Italy (16), supporting the rapid adaptation of these lineages to different hosts and ecosystems.

IncX4 plasmids (~33 kb) were identified by WGS analysis in all strains carrying the *mcr-1* gene. After *de novo* assembly, plasmid sequences were manually annotated using Geneious R9 software, and then PlasmidFinder 1.3 was used to identify incompatibility

TABLE 1 Characteristics of colistin-resistant *Escherichia coli* strains carrying the *mcr-1* gene from Brazil

Characteristic[a]	ICBEC2AM	ICBEC3AM	ICBEC13AM	ICBEC7P	ICBEC72H
Source	Seawater	Seawater	Seawater	Infected migratory penguin	Human infection
Location	−23.974697S, −46.395060W	−23.974995S, −46.371613W	−23.986450S, −46.309086W	−23.986306S, −46.308361W	−5.779257S, −35.200916W
Isolation date	September 2016	September 2016	September 2016	June 2013	March 2016
NaCl tolerance (%)	10	10	10	10	10
Serotype	ONT:H55	O9:H4	O54:H32	ONT:H32	ONT:H9
ST/CC	1638	46/46	10/10	10/10	101/43
Virulence genes	Not detected	*iss*, *gad*, *mchF*	*gad*	*gad*	*iroN*, *mcmA*, *mchB*, *mchC*, *mchF*, *lpfA*, *iss*
Phylogroup	B1	B1	B1	A	B1
Resistance	AMO, AMP, CAZ, CEF, CRO, CTF, CTX, DOX, NAL, SUL, TET	AMO, CEF, CLO, NAL, SUL, SXT	AMO, AMP, ATM, CAZ, CEF, CRO, CTX, DOX, NAL, SUL, SXT, TET	AMK, AMO, AMP, ATM, CAZ, CEF, CIP, CTF, CTX, ENR, FEP, GEN, NAL, SXT, TET	AMO, AMP, ATM, CEF, CTX, FEP
Colistin/polymyxin MIC (μg/ml)	4/4	4/4	4/4	8/8	4/4
Resistance genotype	*mcr-1*, $bla_{CTX-M-8}$, *qnrB19*, *aadA2*, *strA*, *strB*, *sul2*	*mcr-1*, bla_{TEM-1B}, *qnrB19*, *catA1*, *aadA1*, *strA*, *strB*, *sul1*, *sul2*, *tetA*, *dfrA1*, *dfrA8*	*mcr-1*, $bla_{CTX-M-1}$, *aadA1*, *sul2*, *tetA*, *tetB*	*mcr-1*, $bla_{CTX-M-1}$, *aadA1*, *sul2*, *tetA*, *tetB*	*mcr-1*, $bla_{CTX-M-8}$
Plasmids (Inc)[b]	I1, ColRNAI, **X4**	FIB, Q1, **X4**	HI2, I1, N, **X4**	FIN, HI2, HI2A, I1, N, **X4**	I1, **X4**

[a]*E. coli* isolates ICBEC2AM, ICBEC3AM, and ICBEC13AM were analyzed in this study. Data for *E. coli* ICBEC7P and ICBEC72H were obtained from earlier studies by our group (5, 7). ST, sequence type; CC, clonal complex; AMK, amikacin; AMO, amoxicillin; AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CEF, cephalothin; CIP, ciprofloxacin; CLO, chloramphenicol; CRO, ceftriaxone; CTF, ceftiofur; CTX, cefotaxime; DOX, doxycycline; ENR, enrofloxacin; FEP, cefepime; GEN, gentamicin; NAL, nalidixic acid; SUL, sulfonamide; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

[b]The replicon types of plasmids carrying the *mcr-1* gene are in bold.

groups. For comparative analysis, plasmid sequences were aligned against the nonredundant database using the MegaBLAST algorithm (NCBI BLAST), with default settings for the parameters. The plasmids pICBEC2AM and pICBEC3AM displayed 91% and 100% nucleotide identity, respectively, to the plasmid pICBEC72Hmcr (GenBank accession number CP015977), which originated from a human patient (5), and pICBEC13AM displayed 100% identity to the plasmid pICBEC7Pmcr (GenBank accession number CP017246), which was identified in the *E. coli* ST10 isolate from the infected penguin (7), confirming an epidemiological link (Table 1); IncX4 plasmids are key vectors responsible for dissemination of the *mcr-1* gene (5, 7, 17).

The coexistence of *mcr-1* and/or plasmid-mediated quinolone resistance (PMQR) and ESBL-encoding genes, such as *qnrB19* and bla_{CTX-M}-type variants, is of great concern, because the occurrence of *mcr-1* and other clinically significant resistance genes in *E. coli* would seriously compromise treatment options (18). These results suggest that MCR-1-positive *E. coli* isolates are able to recruit other resistance genes, becoming MDR.

In summary, we report the occurrence of colistin-resistant, MCR-1-producing, *E. coli* lineages in recreational coastal waters of anthropogenically affected public beaches (19). In this situation, it is possible that residents, tourists, and wildlife could be exposed to this infectious threat directly from water exposure, from contact with sand, or through food consumption on the beach. Therefore, epidemiological studies addressing the consequences for human health of environmental dissemination of *E. coli* strains carrying the *mcr-1* gene are necessary.

Accession number(s). Complete plasmid sequences were deposited in GenBank under accession numbers KY770023 (pICBEC2AM), KY770024 (pICBEC3AM), and KY770025 (pICBEC13AM).

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We have no conflicts of interest to declare.

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