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SHORT COMMUNICATION

Characterization of *Escherichia* coli strains resistant to carbapenems isolated from rectal swab in a multidrug-resistant strains screening programme

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SUMMARY

The aim of the study is to characterize 28 *Escherichia coli* carbapenem-resistant strains isolated from multi-resistant screening.
All the strains were tested through CARBA NP test and PCR analysis for molecular characterization of carbapenemase. Plasmid characterization and phylogenetic study was performed.
The molecular characterization revealed that 24 of 28 strains harbour carbapenemases.
The most involved plasmids are FIA, FIB, F_{IIS} and F_{repB} replicons that belong to the IncF group.
The phylo-typing analysis revealed a greater presence of the B2 group.
Carbapenem resistance in *E. coli*, should be constantly monitored to avoid the onset of new epidemic episodes.

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Antibiotic resistance is a growing global phenomenon and constitutes a serious health problem at both the clinical and public level. The diffusion of microorganisms resistant to third-generation cephalosporins due to the worldwide spread of ESBLs has caused an increase in the use of carbapenems. Carbapenems are the most powerful β-lactams available, but the onset of carbapenem-hydrolysing enzymes represents a serious concern in gram-negatives, especially in *Enterobacteriaceae* (Livermore, 2009).
There are two main mechanisms underlining carbapenem resistance in *Enterobacteriaceae*: the first is the acquisition of a gene encoding for a carbapenemase; the second is the decrease in the uptake of the antibiotic due to decreased membrane permeability associated with the presence of an ESBL with a weak affinity for carbapenems (Nordman, 2012a).
Hydrolysis of β-lactam antibiotics by β-lactamases is the most common and worrisome mechanism of resistance for this class of antimicrobial drugs in clinically relevant gram-negative bacteria (Bush, 2010).

Key words:
Carbapenem resistance; Carbapenemases; KPC; NDM; VIM

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Among the genes encoding for carbapenemases, in *Enterobacteriaceae* the so called "big-five" are the most widespread. These carbapenemases are categorized in three groups: KPC-types enzymes, metallo-β-lactamases (VIM-, IMP- and NDM-types) and OXA-48.
Carbapenemases in *Enterobacteriaceae* were associated principally with *Klebsiella pneumoniae* and less in *Escherichia coli* (Canton, 2012); but recently *E. coli* resistant to cephalosporins and carbapenems have been noted, although carbapenem-resistant *E. coli* are still rare (ECDC website).
MIC ranges for carbapenems in carbapenemase-producing *Enterobacteriaceae* span from below the susceptible breakpoints to high-level resistance (Livermore, 2009). Therefore, molecular techniques based on PCR followed by sequencing remain the reference standard for the identification of carbapenemases (Nordman, 2012c).
In this study, 28 *E. coli* strains with reduced susceptibility to ertapenem, isolated from rectal swab in the screening of *Enterobacteriaceae* multi-drug resistant surveillance program at the University Verona Hospital, from 2013 to 2017, have been evaluated to check the presence of carbapenemases with phenotypic and genotypic test. The screening programme began in 2013 with about 3000 swab samples and has reached 12,000 annual samples in recent years.
The strains were also characterized for their genetic correlation and for plasmid profile to see if there is a

prevalent clone or plasmid harbouring these enzymes.

Screening was performed by streaking the rectal swab on ChromoID ESBL agar plate (Biomerieux) plus an ertapenem disk (10 µg). Identification was confirmed by MALDI-tof using VITEK-MS instrument (Biomerieux). Strains with a halo for Ertapenem less than 25 mm were selected for the study.

Broth Microdilution method and E-TEST method were used for MIC determination for carbapenems (ertapenem, meropenem and imipenem), cephalosporins (ceftazidime, cefotaxime, cefepime and ceftazidime-avibactam), aminoglycosides (gentamicin and amikacin), ciprofloxacin and colistin. To test ceftazidime-avibactam, a constant concentration of 4 mg/L was used for avibactam (Sigma, srl). The EUCAST guidelines (https://www.eucast.org/clinical_breakpoints/table.11) were used for MIC interpretation.

Phenotypic detection of carbapenemases was performed. Carba NP test is a rapid, sensitive and specific test to identify hydrolysis of the β-lactam ring of a carbapenem (imipenem) due to the presence of a carbapenemase (Nordman, 2012b).

Molecular characterization of carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM} and bla_{OXA-48}) and ESBL of CTX-M groups were performed through Polymerase Chain Reaction (PCR) (Dallenne, 2010). Complete Bla_{NDM} and bla_{VIM} genes were amplified and sequenced (Mazzariol, 2012; Mazzariol, 2011).

Plasmid profiling was performed using the Carattoli protocol (2005). The technique consists of 5 multiplex and 3 simplex PCR reactions using eighteen pairs of primers designing to recognize HI1, HI2, X, L/M, N, FIA, FIB, W, Y, P, FIC, A/C, T, FII_S, K, B/O and F_{repB} replicons, representative of the major plasmid incompatibility group among *Enterobacteriaceae*.

Phylogenetic *E. coli* analysis was performed with the Clermont Phylo-Typing analysis (Clermont, 2013). The technique consists of a Multiplex PCR reaction to detect genes *chuA* (outer membrane hemin receptor), *yjaA* (stress response protein), TspE4.C2 (putative lipase esterase gene) and *arpA* (ankiryn-like regulatory protein). Based on the obtained genotype, the technique allows *E. coli* strains to be assigned to seven different phylo-groups (A, B1, B2, C, D, E, F).

Out of 28 total strains studied, 24 isolates (85.7%) were identified as carbapenemase-producers through CARBA NP test.

Susceptibility data are shown in *table 1*. 100% of identified carbapenemase-producing strains showed resistance to ertapenem, 33.3% (8 out of 24 isolates) showed resistance to imipenem, while 12.5% (3 out of 24 isolates) showed reduced susceptibility to meropenem.

These data confirm the difficulty in detecting carbapenemases in clinical specimens, where automated systems are often used. Automated systems may not be reliable to detect all types of carbapenemase-producers. Ertapenem seems to be a good candidate to detect the presence of carbapenemase, since MICs of ertapenem are usually higher than MICs of other carbapenems (Nordman, 2012b). Our work also included strains with MICs for Ertapenem of 0.5 and 0.25 µg/ml.

Since 2018, EUCAST breakpoints for imipenem and ertapenem have been lowered to permit better detection of carbapenem-resistant isolates and, even for meropenem, EUCAST suggests using a cut-off higher than 0.125 µg/ml for carbapenemase screening, underlining the possibility that some carbapenemase-producers may be susceptible to the current carbapenem breakpoints (https://www.eucast.org/clinical_breakpoints/table.8)

The increased reliance on carbapenems in the treatment of gram-negative infections has led to the onset of carbapenems-resistant pathogens. Therapeutic strategies for the treatment of infections caused by carbapenems-resistant pathogens involve the administration of combined penicillins or cephalosporins with β-lactamase-inhibitors or the administration of colistin, an old antibiotic re-introduced as the last therapeutic resource due to its nephrotoxic and neurotoxic activity. All the strains in this study were completely susceptible to colistin with MIC ranges lower than 0.125 µg/ml, while 24 out of 28 tested strains were susceptible to ceftazidime-avibactam.

The PCR analysis data, reported in table 1, reveals 24 isolates out of 28 harbour a carbapenemase gene: 71.4% (20 isolates out of 28) harbour bla_{KPC}, while 4 strains harbour metal-β-lactamase genes, namely two bla_{NDM} and two bla_{VIM}; corresponding to the strains showing ceftazidime-avibactam resistance.

Ceftazidime-avibactam is inactive toward these enzymes, and so it is normal to find these strains resistant. It is worth noting that this combination of cephalosporin and beta-lactamases inhibitor is still active toward class A carbapenemases as KPC, including when present in *E. coli* strains.

The BLAST analysis for NDM-producers revealed that both these isolates harbour the NDM-5 carbapenemase. Although NDM enzymes are rarely detected in Italy, cases of NDM-5-producing *E. coli* have been reported (Giufrè, 2018) and an outbreak of NDM-producing Enterobacteria is still ongoing in Tuscany (Italy) (Tavoschi, 2020). VIM-producer strains harbour VIM-1. This enzyme was first recovered in *Pseudomonas aeruginosa* strains in Verona (Italy) (Lauretti, 1999) and is now also found in *E. coli*. The CARBA NP test correctly identified all the carbapenemase-producers, but PCR was needed to characterize the type of carbapenemase, because MBLs are the major concern at the clinical level due to the presence, in their active site, of zinc ions, which make these enzymes resistant to all β-lactamases-inhibitors. CarbaNP is an important tool in the screening, since unknown car-

Table 1 - *Antibiotic susceptibilities, CarbaNP test, beta-lactamase genes, plasmids and phylogenetic profile of E. coli strains under study.*

Strains	MICs (mg/L)										CarbaNP test	Carbapenemase	CTX-M group	Plasmids	Philogenetic profile
	ERT	IMP	MPM	CPM	CAZ	CZA	AMI	GEN	CIP	COL					
mdr219	1	4	2	64	>128	>128	4	4	>128	<0.06	pos	VIM	none	I1, N, FIB, P, FREP B	B2
mdr600	8	8	8	32	>128	1	4	0.25	>128	<0.06	pos	KPC	none	FIA, FIB, F IIS,	D or E
mdr1696	4	8	2	>128	128	0.5	4	2	>128	<0.06	pos	KPC	M1	XFIA, FIB, FIIS	B2
mdr1997	4	2	1	8	>128	2	2	64	>128	<0.06	pos	KPC	none	I1, FIB, O, FIIS, B/O FREP B	A
mdr2114	2	2	0.5	16	>128	1	4	2	<0.06	<0.06	pos	KPC	none	N, FIB, P, FREP B	B2
mdr2225	2	2	1	16	>128	0.5	2	0.5	128	<0.06	pos	KPC	none	N, FIA, FIB, FIIS, FREP B	B2
mdr2247	2	4	1	8	64	1	8	2	>128	<0.06	pos	KPC	none	I1, FIA, Y, FIIS, FREP B	F
mdr2270	2	4	1	16	>128	2	2	0.25	64	<0.06	pos	KPC	M9	FIA, FIB, FIIS, FREP B	B2
mdr2367	4	4	2	64	128	0.5	16	0.5	>128	<0.06	pos	KPC	none	FIA, FIB, Y, FIIS, FREP B	D or E
mdr2440	2	4	1	128	>128	2	4	1	64	<0.06	pos	KPC	M1	FIA, FIB, FII S, FREP B	B2
mdr2442	1	2	0.5	>128	>128	0.5	2	0.5	32	<0.06	pos	KPC	M1	FIA, FIB, FII S, FREP B	B2
mdr2664	4	2	0,5	8	64	1	32	1	>128	<0.06	pos	KPC	none	I1, FIA, FIB, Y, FIIS, FREPB	D or E
mdr2984	4	8	1	16	>128	0.25	4	0.5	4	<0.06	pos	KPC	none	FIB, P	A
mdr3361	4	1	0,5	16	>128	4	1	0,25	128	<0.06	neg	-	none	ND	ND
mdr4467	1	0.5	05	32	32	0.5	4	0.5	<0.06	<0.06	neg	-	M1	ND	ND
mdr4517	4	4	2	8	32	0.25	8	1	128	<0.06	pos	KPC	none	FIA, FIB P, Y, FII, F REP B	B2
mdr4783	2	4	2	16	>128	1	2	1	32	<0.06	pos	KPC	none	FIA, FIB, P, FIIS, FREP B	B2
mdr4834	0.25	0.12	<0.06	1	128	0.25	2	1	<0.06	<0.06	neg	-	none	ND	ND
mdr4909	2	4	2	16	64	0.12	4	1	<0.06	<0.06	pos	KPC	none	FIB, FII S, FREP B	B2
mdr9135	4	4	1	16	128	1	2	0.5	<0.06	<0.06	pos	KPC	M1	FIA, FIB, P, FII S, FREP B	A
mdr9139	2	4	1	8	64	0.5	2	1	<0.06	<0.06	pos	KPC	none	FII S	A
mdr9140	1	4	0,25	>128	>128	0.5	4	0.5	128	<0.06	pos	KPC	M1	FIB, FII S, FREP B	B2
mdr9141	64	16	64	>128	>128	>128	1	0.25	64	0.12	pos	NDM	M1	I1, FIA, FIB, W, Y, FREP B	A
mdr9144	8	8	4	128	>128	2	1	1	64	<0.06	pos	KPC	none	FIA, FIB, P, FII S	B2
eco 9145	0.5	0.25	<0.06	>128	>128	2	4	1	>128	<0.06	neg	-	M1	ND	ND
mdr9147	>128	>128	>128	>128	>128	>128	16	1	64	<0.06	pos	VIM	none	FIA, FIC,	D or E
mdr9148	16	16	8	>128	>128	1	2	0.5	64	<0.06	pos	KPC	none	N, FIA, FIB, FII S, FREP B	B2
mdrNDM	128	16	64	>128	>128	>128	1	0.25	128	<0.06	pos	NDM	M1-M9	FIA, FIB, P, FREP B	A

ERT, ertapenem; IMI, imipenem; MPM, meropenem; CPM, cefepime; CAZ, ceftazidime; CZA, ceftazidime-avibactam; AMI, amikacin; GEN, gentamicin; CIP, ciprofloxacin; COL, colistin;
ND: not determined

bapenemases or carbapenemases produced by strains with low susceptibilities could also be detected.

PCR data analysis of the *bla* $_{CTX-M}$ group showed that nine strains out of 28 harbour the M1 group and two strains harbour the M9 group.

PBRT analysis data, reported in *Table 1*, show that the most involved plasmids are FIA (70.8%), FIB (92%), F_{IIS} (75%) and F_{repB} (75%) replicons that belong to IncF.

All strains harbour at least one of the replicons belonging to this family. F plasmids are highly associated with antibiotic resistance and are the most abundant plasmid type in *Enterobacteriaceae* (Partridge, 2018). IncF plasmids have been associated with the spread of serine carbapenemase KPC, plasmid-mediated AmpC, quinolone and aminoglycoside resistance genes (Carattoli, 2009; Carattoli, 2013). Our results confirm the association of KPC carbapenemase with the IncF plasmid family not only in *K. pneumoniae* strains but also in *E. coli*, contributing to the spread of this plasmid. The problem is that this mechanism could not be detected in *E. coli* due to its low carbapenems MICs value, often below the breakpoint.

The Clermont Phylo-Typing analysis showed that 54.1% (13 out of 24 isolates) were identified with the B2 profile, which is most associated with *E. coli* involved in extra-intestinal infections (Clermont, 2013). The high-risk clone *E. coli* ST131 belongs to the B2 Clermont Phylo-Type profile (Furlan, 2020). This high-risk clone KPC-2 producer emerged in China (Cai, 2014). It is worth noting that B2 also seems to correlate with the presence of an ESBL in the CTX-M group. As a future goal, it will be interesting to perform MLST to determine the sequence type of these strains belonging to the B2 phylo-type group and its association with other resistance determinant and virulence factors. The predominance of the B2 phylo-type *E. coli* harbouring carbapenemases and the low carbapenems MICs of these strains is a source of worry.

Also worth noting is the presence of different phylo-type groups, such as A, F and D/E.

Carbapenem resistance in *E. coli* and in *Enterobacteriaceae* should be constantly monitored to avoid the onset of new epidemic episodes in hospitals and the community. The most recent epidemiological data must be evaluated to establish the trends of this phenomenon.

Conflicts of interests

All authors declare no conflicts of interest in this paper.

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