

LETTER TO THE EDITOR

New Delhi metallo- β -lactamase and OXA-48 carbapenemases in Gram-negative bacilli isolates in Libya

To the Editor

Carbapenems (i.e. ertapenem, imipenem, meropenem, and doripenem) are second line antimicrobials reserved to treat serious infections, including those caused by Gram-negative bacilli (GNB) producing extended-spectrum β -lactamases (ESBLs). ESBLs mediate resistance among GNB to β -lactam drugs, including third generation cephalosporins. Emergence of carbapenemases-producing GNB (mainly *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and members of the family *Enterobacteriaceae*) in the last decade is a serious health problem globally. Carbapenemases are members of three molecular groups of β -lactamases, Ambler class A, B (i.e. metallo β -lactamases [MBLs]), and D (i.e. oxacillinases) (1, 2). Recently, the novel carbapenemase New Delhi MBL (NDM) was reported for the first time in Tunisia in *Klebsiella pneumoniae* isolated from a female patient transferred from Libya (3). However, there is little information regarding genes associated with carbapenem resistance among GNB in Libya.

Included in the study were eight GNB isolates that are resistant to at least one carbapenem (i.e. ertapenem) obtained in 2013 and 2014 from different clinical specimens from patients at the time an infection occurred. Patients were aged between 10 and 87 years (mean = 37.1 years). Information about patients and the organisms isolated from them included in the study are shown in Table 1. In this prospective investigation, clinical specimens were collected under approved ethical standards, and the study was reviewed and approved by the Academy of Graduate Studies, Tripoli, Libya.

All specimens were cultured on plates of blood agar and MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37°C for 24–48 h. Isolated organisms were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents by the BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks, MD, USA) as recommended by the manufacturer. PAMS provides antimicrobial susceptibility results as susceptible (S), intermediate susceptible (I), or resistant (R) and are interpreted according to Clinical and Laboratory Standards Institute criteria (4). Quality control strains used included *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 (ATCC, LGC Standards S.r.l., Italy). Furthermore,

six of the GNB isolates were tested phenotypically for the production of MBLs by the MIC Test Strip MBL (Liofilchem, Rosetodegli Abruzzi, Italy) as recommended by the manufacturer.

All eight isolates were examined for genes encoding ESBLs *bla*_{CTX-M}, *bla*_{GES}, *bla*_{VEB}, *bla*_{PER}, or *bla*_{BES} and carbapenemases *bla*_{KPC}, *bla*_{VIM}, *bla*_{NDM}, or *bla*_{OXA-48} by polymerase chain reaction (PCR) using primers and conditions described previously (5, 6). In addition, both *A. baumannii* isolates were further tested for carbapenemase genes *bla*_{OXA-23-like}, *bla*_{OXA-24/40-like}, *bla*_{OXA-143-like}, *bla*_{OXA-51-like}, and *bla*_{OXA-58-like} using PCR as above.

All *A. baumannii* and *Pseudomonas* sp. isolates were resistant to the three carbapenems as well as to nearly all other antimicrobials that were used. Only colistin showed excellent activity against the GNB isolates investigated (100% susceptible) followed by amikacin (50% susceptible). Furthermore, all organisms were multidrug resistant (MDR; resistance to three or more antimicrobial groups). Table 2 shows the antimicrobial susceptibility of the investigated GNB isolates.

Of the ESBLs genes examined, *bla*_{CTX-M} was detected in one *K. pneumoniae* and in the *Enterobacter gergoviae* isolate, and *bla*_{GES} in the *P. aeruginosa* isolate. Other ESBL genes examined were not detected in all eight GNB isolates. Of the carbapenemase genes investigated, *bla*_{NDM} was detected in one *K. pneumoniae*, and *bla*_{OXA-48} in *E. gergoviae* and in another *K. pneumoniae* isolate. On the other hand, the carbapenemase gene *bla*_{KPC} was not detected in all isolates investigated. Table 3 shows genes encoding ESBLs and carbapenemases in the GNB isolates from Tripoli, Libya.

Oxacillinases are mainly present in *Acinetobacter* sp. (7), and some of them are intrinsic to the organism (e.g. OXA-51) while others are acquired (e.g. OXA-23). *bla*_{OXA-23-like} and *bla*_{OXA-51-like} were found in both *A. baumannii* isolates. Recently, Mathlouthi et al. (8) reported MDR *A. baumannii* isolates harboring *bla*_{OXA-23-like}, *bla*_{OXA-24-like}, and *bla*_{OXA-48-like} genes in Libya.

On the other hand, we identified OXA-48 in one *K. pneumoniae* as well as in the *E. gergoviae* isolate. Kocsis et al. (9) reported *K. pneumoniae* harboring OXA-48 carbapenemase in a Libyan refugee in Italy. In addition, several studies reported OXA-48 carbapenemase-producing *K. pneumoniae* in patients transferred from

Table 1. Information about patients and their isolated organisms

Patient	Gender	Age (years)	Hospital/department	Hospital admission	Specimen	Organism isolated
1	Male	30	TMC/ICU	Inpatient	Endotracheal tube tip	<i>Acinetobacter baumannii</i>
2	Female	57	TMC/GSICU	Inpatient	Suction tip	<i>Acinetobacter baumannii</i>
3	Female	46	TMC/ICU	Inpatient	Sputum	<i>Pseudomonas aeruginosa</i>
4	Male	25	BPSH/ICU	Inpatient	Wound swab	<i>Pseudomonas putida</i>
5	Male	10	BPSH	Outpatient	Wound swab	<i>Escherichia coli</i>
6	Male	87	TMC	Outpatient	Ear swab	<i>Klebsiella pneumoniae</i>
7	Female	42	TMC/Endo	Inpatient	Bedsore swab	<i>Klebsiella pneumoniae</i>
8	Male	30	TMC/GSICU	Inpatient	Wound swab	<i>Enterobacter gergoviae</i>

TMC = Tripoli Medical Center, BPSH = Burn and Pediatric Surgery Hospital, ICU = intensive care unit, GSICU = general surgical ICU, OP = outpatient, Endo = endocrinology. TMC and BPSH are both located in Tripoli.

Table 2. Antimicrobial susceptibility and metallo- β -lactamases of Gram-negative bacilli isolates from different clinical specimens in Tripoli, Libya

No.	Organism	Antimicrobial agent																
		AM	CN	EP	IP	MP	CX	CR	CT	CZ	CP	AN	PT	TS	CF	LF	CL	MBLs
1	<i>Acinetobacter baumannii</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	+
2	<i>Acinetobacter baumannii</i>	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	ND
3	<i>Pseudomonas aeruginosa</i>	R	R	R	R	R	R	R	R	I	I	I	S	R	R	R	S	+
4	<i>Pseudomonas putida</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	+
5	<i>Escherichia coli</i>	S	R	R	S	S	R	R	R	S	R	R	S	R	R	R	S	-
6	<i>Klebsiella pneumoniae</i>	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	+
7	<i>Klebsiella pneumoniae</i>	S	R	R	I	R	R	R	R	R	R	R	S	R	R	S	-	
8	<i>Enterobacter gergoviae</i>	S	R	R	S	R	R	R	R	R	R	R	S	R	R	S	ND	

AK = amikacin, CN = gentamicin, EP = ertapenem, IP = imipenem, MP = meropenem, CX = ceftazidime, CR = cefuroxime, CT = ceftriaxone, CZ = ceftazidime, CP = cefepime, AN = aztronam, PT = piperacillin-tazobactam, TS = trimethoprim-sulfamethoxazole, CF = ciprofloxacin, LF = levofloxacin, CL = colistin. S = susceptible, I = intermediate susceptible, R = resistant, MTLs = metallo β -lactamases tested phenotypically, + = positive, - = negative, ND = not done.

Table 3. Genes encoding extended-spectrum β -lactamases (ESBLs) and carbapenemases in Gram-negative bacilli from Tripoli, Libya

No.	Organism	ESBLs genes ^a		Carbapenemase genes ^{a,b}				
		<i>bla</i> _{CTX-M}	<i>bla</i> _{GES}	<i>bla</i> _{NDM}	<i>bla</i> _{VIM}	<i>bla</i> _{OXA-48}	<i>bla</i> _{OXA-23-like}	<i>bla</i> _{OXA-51-like}
1	<i>Acinetobacter baumannii</i>	N	N	N	N	N	P	P
2	<i>Acinetobacter baumannii</i>	N	N	N	N	N	P	P
3	<i>Pseudomonas aeruginosa</i>	N	P	N	N	N	ND	ND
4	<i>Pseudomonas putida</i>	N	N	N	P	N	ND	ND
5	<i>Escherichia coli</i>	N	N	N	N	N	ND	ND
6	<i>Klebsiella pneumoniae</i>	P	N	P	N	N	ND	ND
7	<i>Klebsiella pneumoniae</i>	N	N	N	N	P	ND	ND
8	<i>Enterobacter gergoviae</i>	P	N	N	N	P	ND	ND

^aESBLs genes *bla*_{VEB}, *bla*_{PER} and *bla*_{BES} and carbapenemase gene *bla*_{KPC} were not detected in all eight isolates tested.

^bCarbapenemases genes *bla*_{OXA-24/40-like}, *bla*_{OXA-58-like} and *bla*_{OXA-143} were not detected in both *A. baumannii* isolates examined.

N = negative, P = positive, ND = not done.

Libya to neighboring and European countries (e.g. Tunisia, Denmark, and Slovenia) (3, 10–12). However, there are no reports of *bla*_{OXA-48}-positive *K. pneumoniae* and *E. gergoviae* from Libya.

Both *bla*_{CTX-M} and *bla*_{NDM} were detected in *Klebsiella pneumoniae* isolated from an ear swab from a female outpatient with an ear infection. This isolate was also positive for MBLs by the phenotypic method used (Table 2). *K. pneumoniae* harboring *bla*_{NDM} has not been reported from Libya in the past.

Genes coding for the ESBLs and carbapenemases tested were not detected in the *E. coli* isolate. However, the organism was ESBL-positive phenotypically (data not shown), which may suggest that other ESBLs not investigated in this work might be responsible for its resistance to the third generation cephalosporin.

In conclusion, to the authors' knowledge this is the first report from Libya of *K. pneumoniae* harboring *bla*_{NDM} and *bla*_{CTX-M}, *K. pneumoniae* harboring *bla*_{OXA-48}, and *E. gergoviae* harboring *bla*_{OXA-48} and *bla*_{CTX-M}. Emergence of MDR GNB harboring genes coding for carbapenemases will undoubtedly limit the use of carbapenems in treating serious infectious in the country and also in the nearby countries.

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