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First Report of German Cockroaches (*Blattella germanica*) as Reservoirs of CTX-M-15 Extended-Spectrum-β-Lactamase- and OXA-48 Carbapenemase-Producing *Enterobacteriaceae* in Batna University Hospital, Algeria

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Here we report the isolation of extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing *Enterobacteriaceae* from German cockroaches caught in the burn unit of Batna University Hospital in Algeria. Nine of 12 isolates harbored the $bla_{CTX-M-15}$ ESBL gene. One *Enterobacter cloacae* isolate belonging to sequence type 528 coexpressed the bla_{OXA-48}, $bla_{CTX-M-15}$, and bla_{TEM} genes. Our findings indicate that cockroaches may be one of the most dangerous reservoirs for ESBL and carbapenemase producers in hospitals.

The emergence of multidrug-resistant *Enterobacteriaceae* (MDRE) and their dissemination in hospitals have become a major health concern worldwide (1). The production of β-lactamases such as extended-spectrum β-lactamase (ESBL) and carbapenemase remains the main mechanism of resistance in *Enterobacteriaceae* (2). Algeria is now feaures among the Mediterranean countries known to be affected by the spread of CTX-M-15 type ESBL and the emergence of the phantom menace OXA-48 type carbapenemase (3, 4). Indeed, the transmission and dissemination of MDRE in hospitals are usually related to hand contact (5) and poorly sterilized instruments (6). However, cockroaches which colonize these environments may also act as potential mechanical vectors of antibiotic-resistant bacteria (7). Cockroaches are among the most prevalent pests in hospitals, where they are attracted by moisture, food, and suitable temperatures (8, 9). The medical significance of cockroaches has been largely overlooked, but it has been proven that they can harbor a number of pathogenic microorganisms with different levels of antibiotic resistance (10). *Blattella germanica* is the most abundant cockroach species (11), and the external surfaces and excrement of these cockroaches can contaminate food, human habitats, and hospital equipment; therefore, these insects can present a threat to human health (12).

The aim of our study was to screen for broad-spectrum-cephalosporin- and carbapenem-resistant *Enterobacteriaceae* in *B. germanica* cockroaches collected from the burn unit of Batna University Hospital in Algeria and then to investigate the molecular support of ESBL and carbapenemase production.

In March 2015, 10 cockroaches were randomly captured from different parts of the burn unit, including the kitchen and the guard, treatment, and patient rooms, directly in sterile containers. Samples were immobilized by freezing at 0°C for 10 min (8). Each cockroach was soaked in 5 ml of Tween 80 solution at 0.05% and vortexed vigorously for 2 min. The resulting wash was used as an external-body-homogenate sample. To remove the external body contamination, cockroaches were immersed in bleach for 2 min, in sterile physiological saline for 2 min, and then in 70% ethanol for 5 min (12). Then, each sample was washed with sterile physiological saline. Subsequently, the insect was soaked in a sterile bottle containing 5 ml of Tween 80 solution at 0.05% and then crushed inside using a sterile pestle. The triturate was then vortexed vigorously for 2 min. The resulting suspension was used as an internal-body-homogenate sample. Each sample (5 ml) was preenriched for 4 h in 5 ml of a double concentration of brain heart infusion (BHI) (BD: Becton, Dickinson and Company, France). Subsequently, the screening for β-lactam-resistant *Enterobacteriaceae* began with a selective enrichment step using two selective media: BHI supplemented with 64 mg/liter of vancomycin and 1 mg/liter of ertapenem and BHI supplemented with 64 mg/liter of vancomycin and 2 mg/liter of cefotaxime. After overnight incubation at 37°C, cultures were inoculated by streaking 100 μl of the growth onto MacConkey agar plates (BD) with 64 mg/liter of vancomycin supplemented with 1 mg/liter of ertapenem or 2 mg/liter of cefotaxime, respectively. The obtained *Enterobacteriaceae* isolates were identified using an API 20E system (bioMérieux, France) and confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (13). Susceptibility testing was performed using a standard disk diffusion technique according to the recommendations of the Antibiotic Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_2015.pdf). ESBL detection was per-

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formed using the double-disk synergy test (DDST) as previously described (14). The modified Hodge test (MHT) and the modified Carba NP (MCNP) test were used to screen for carbapenemase activity (15, 16). All strains were screened using real-time PCR for the presence of the following β-lactamase-encoding genes: bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{CTX-M}, bla_{TEM}, and bla_{SHV} (17–19). Strains positive by real-time PCR were subjected to standard PCR and sequencing (18, 20). Multilocus sequence typing (MLST) was carried out for carbapenemase-producing strains with the PubMLST scheme (http://pubmlst.org/ecloacae/).

Twelve *Enterobacteriaceae* isolates were obtained from 12 representative colonies, and strains of the following species were identified: *Klebsiella oxytoca*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Citrobacter amalonaticus* (Table 1). The isolation from hospital cockroaches of such *Enterobacteriaceae* species with different levels of antimicrobial resistance has already been described (21). Additionally, in this study, we report for the first time the identification of the *Enterobacteriaceae* species *Citrobacter farmeri*, *Citrobacter koseri*, and *Enterobacter kobei* in German cockroaches. The majority of strains were isolated from the internal organs (Table 1), which could be explained by the great adaptation of these organisms in the intestinal environment (22). The antimicrobial susceptibility test results are presented in Table 1. The DDST was positive for 11 of 12 isolates. The MHT and the MCNP test were positive for only one *E. cloacae* isolate. Eleven of the 12 obtained strains harbored bla_{TEM}, and 75% (n = 9) of all isolates coharbored the $bla_{CTX-M-15}$ ESBL gene. One *E. cloacae* isolate belonging to sequence type 528 (ST528) coharbored the bla_{OXA-48}, $bla_{CTX-M-15}$, and bla_{TEM} genes. According to the antibiogram results, some of the strains could express other β-lactamases not sought in this study. To our knowledge, this is the first description of CTX-M-15-producing *Enterobacteriaceae* isolated from *B. germanica* cockroaches. Few reports describing the presence of ESBL-producing *Enterobactericeae* in hospital cockroaches have been published (23–26). The most interesting finding in the present study was the first detection of OXA-48-producing *E. cloacae* in *B. germanica*. Since its first description in Turkey (27), numerous reports have indicated the spread of OXA-48 type carbapenemase in Mediterranean countries (28–30), where it represents the most common carbapenemase type (31). Subsequently, several studies have reported the isolation of OXA-48-producing *Enterobacteriaceae* from different samples (32, 33). Prior to its first description in Algeria, Poirel et al. suggested that OXA-48 may be endemic in this country (34). The huge spread of CTX-M-15 ESBL-producing *Enterobacteriaceae* and the emergence of OXA-48-type carbapenemase-producing *Enterobacteriaceae* in Algerian hospitals may explain the carriage of such drug-resistant bacteria by hospital cockroaches through their particular feeding habits (3, 4, 35).

The important role of cockroaches as a potential reservoir of multidrug-resistant bacteria demonstrated in this study and in earlier investigations with their presence in hospital environments, which has already been reported in Algeria (36) and other countries such as Turkey (9), Japan (37), and Cuba (38), urgently call for regulations regarding their control and elimination from clinical areas (11).

TABLE 1 Results of species identification and antibacterial susceptibility tests and β-lactamase determinants

Strain code	Source/ location[a]	Species identification	Antibiotic resistance[b]															β-lactamase-encoding genes
			AMX	AMC	FOX	CTX	CAZ	FEP	ATM	ETP	IPM	TOB	GEN	AMK	CIP	SXT	TGC	CST	
S1	IO/K	*Citrobacter amalonaticus*	R	S	S	R	R	R	R	S	S	I	R	S	S	R	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S2	ES/P	*Citrobacter amalonaticus*	R	R	R	R	R	S	R	S	S	R	R	S	S	R	S	S	bla_{TEM}
S3	IO/K	*Citrobacter farmeri*	R	R	R	R	R	S	R	S	S	R	R	S	S	R	S	S	bla_{TEM}
S4	IO/T	*Citrobacter freundii*	R	R	R	R	R	R	R	R	S	R	R	S	R	S	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S5	IO/K	*Citrobacter koseri*	R	S	S	R	R	R	R	S	S	I	R	S	S	R	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S6	IO/T	*Enterobacter cloacae*	R	R	R	R	R	R	R	R	S	R	R	S	I	S	S	S	bla_{OXA-48}, $bla_{CTX-M-15}$, bla_{TEM}
S7	IO/T	*Enterobacter cloacae*	R	R	R	R	R	R	R	S	S	R	R	S	I	R	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S8	ES/P	*Enterobacter cloacae*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S9	ES/T	*Enterobacter kobei*	R	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S	None
S10	IO/T	*Klebsiella oxytoca*	R	S	S	R	R	R	R	S	S	R	R	S	S	R	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S11	IO/K	*Klebsiella oxytoca*	R	S	S	R	R	I	I	S	S	R	R	S	S	S	S	S	$bla_{CTX-M-15}$, bla_{TEM}
S12	IO/K	*Klebsiella oxytoca*	R	S	S	R	R	R	R	S	S	R	R	S	S	S	S	S	$bla_{CTX-M-15}$, bla_{TEM}

[a] ES, external surface; IO, internal organs; K, kitchen; P, patient's room; T, treatment room.

[b] AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; ETP, ertapenem; IPM, imipenem; TOB, tobramycin; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; CST, colistin; R, resistant; S, susceptible; I, intermediate.

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