JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Molecular characterization and diversity of carbapenemases in Gram-negative bacteria in Libyan hospitals

Khouloud Slimene[1,2,3,4,5], Salem K Almahjoub[6], Aldukali A Alkeskas[7], Ahmed I Elbousify[8], Elham O Omar[9], Allaaeddin A El Salabi[10], Elhussan A Mohamed[11], Mohammed I Hameid[12], Souad Alsanosi[12], Dhouha Miniaoui[1,2,3,4,5], Jean-Marc Rolain[1,2], Chedly Chouchani[3,4,5]

[1] *Université Aix-Marseille, IRD, APHM, MEPHI, Faculté de Médecine et de Pharmacie, France*
[2] *IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13385 Marseille Cedex 05, France*
[3] *Laboratoire des Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Campus Universitaire, Tunisia*
[4] *Laboratoire de Recherche des Sciences et Technologies de l'Environnement, Institut Supérieur des Sciences et Technologies de l'Environnement de Borj-Cédria, Université de Carthage, Tunisia*
[5] *Unité de Service en Commun pour la Recherche, Plateforme Génomique, Institut Supérieur des Sciences et Technologies de l'Environnement de Borj-Cédria, Université de Carthage, Tunisia*
[6] *Department of Epidemiology and Infection Control, Faculty of Health Sciences, Misurata University, Misurata, Libya*
[7] *Department of Microbiology, Faculty of Science, Misurata University, Misurata, Libya*
[8] *Department of Pediatrics, Faculty of Medicine, University of Tripoli, Tripoli, Libya*
[9] *Department of Medical Microbiology, Faculty of Medicine, University of Benghazi, Benghazi, Libya*
[10] *Department of Public Health, Faculty of Medical Technology, University of Tripoli, Tripoli, Libya*
[11] *Central Sterilization Service Department, Benghazi Medical Center, Benghazi, Libya*
[12] *Infection Control Office, Al-Jalah Trauma Hospital, Benghazi, Libya*

Abstract

Introduction: Antimicrobial resistance has become a major threat to public health, especially in developing countries, due to the uncontrolled consumption of antibiotics. This study aims to characterize antibiotic resistance genes in different bacteria recovered in different healthcare facilities in Libya.

Methodology: 379 samples were recovered from various sources from different sites. 210 samples were able to grow on culture media. 133 Gram-negative carbapenem-resistant strains were recovered from clinical specimens (n = 64), and hospital environments (n = 69). Antibiotic susceptibility tests were performed to select carbapenem-resistant strains. Colistin resistance was tested by the UMIC method to determine the minimum inhibitory concentration. RT-PCR was conducted to detect the incidence of carbapenemases-encoding genes.

Results: Gram-negative bacteria showed a low susceptibility to carbapenems. Molecular investigations indicated that NDM-1 was the most prevalent in *Enterobacteriaceae* isolated from patients and hospital environment (n = 26, n = 41), followed by $bla_{\text{OXA-48}}$ (n = 16, n = 15) and bla_{VIM} (n = 3) from patients and bla_{KPC} (n = 1) from hospital environment. Concerning *A. baumannii*, bla_{OXA-23} was detected in strains isolated from patients (n = 8) and hospital environment (n = 6), followed by bla_{NDM} (n = 9) from patients and one from hospital environment. Carbapenem resistance in *P. aeruginosa* was encoded by modification in OprD encoding gene, such as *IS* (*IS*pa26), polymorphism, and a premature stop codon.

Conclusions: Several carbapenem resistant Gram-negative bacteria were identified by the expression of different carbapenemases and the alteration of OprD.

Key words: Gram-negative bacteria; antimicrobial resistance; carbapenemase; clinical specimens; hospital environment; Libya.

J Infect Dev Ctries 2025; 19(7):1089-1099. doi:10.3855/jidc.18674

(Received 02 June 2023 – Accepted 14 November 2023)

Introduction

Contaminated hospital environments that lack infection control and prevention measures have been found to have a role in the spread of nosocomial pathogens [1]. Numerous studies have demonstrated that the hospital environment can facilitate the transmission of nosocomial pathogens to patients, they include; methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and *Clostridium difficile*, that are able to survive for months on dry surfaces [2]. Several Gram-negative species, like *Acinetobacter* spp, *Escherichia coli*, *Klebsiella* spp, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Shigella* spp. can persist on inanimate surfaces for months [3] and were isolated from infected patients [4]. These bacteria persist in a hospital environment, particularly in the environment surrounding colonized or infected patients. These

micro-organisms can remain in the environment for days and, in some cases, months, their circulation is facilitated by healthcare personnel (ref). Some Gram-negative bacteria have been described to persist longer than Gram-positive bacteria [5]. Antibiotics are used to treat bacterial infections in hospitals and are considered a major source of antibiotic-resistance determinants [6]. The spread of antimicrobial-resistant bacteria (AMRB) is considered a worldwide issue and has become a global threat to public health. AMRB is identified by the World Health Organization (WHO), the European Union (EU), the United States Government (US), and Centre for Disease Control and Prevention (CDC) as one of the most significant threats to human health [7]. High levels of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARG) have been reported in hospital wastewater, even after wastewater treatment [8,9]. Furthermore, the inanimate environment can serve as a reservoir for multi-drug-resistant bacteria (MDRB) [10,11]. It has been found that environmental contamination is responsible for 10 - 30% of MDRB infections in patients and is often responsible for increasing morbidity and mortality rates due to the limited availability of treatment options [12].

Several antibiotics used to treat infections caused by Gram-negative bacteria are affected by enzymes produced by chromosomally and/or plasmid-mediated antibiotic resistance genes. The most spread enzymes include Extended Spectrum Beta-Lactamases (ESBLs), AmpC enzymes, Metallo- β -lactamases (MBLs), and carbapenem Hydrolysing class D β-lactamases (CHDLs). In view of the emergence of ESBLs and AmpC-producing Gram-negative bacteria, carbapenems (imipenem, meropenem, and ertapenem), have shown their stability for treating infections due to multi-drug-resistant bacteria, mainly Gram-negative bacterial infections [13]. Nevertheless, those bacteria have the strongest impact on the development and emergence of antimicrobial resistance, both as fermenters (*Enterobacteriaceae*) and non-fermenters of Gram-negative bacilli (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) [14].

Two principal mechanisms are responsible for resistance to carbapenem antibiotics: (i) Production of ESBL and/or AmpC enzymes with non-significant carbapenemase activity, combined with a loss of porin or up-regulation of efflux pump (ii) Secretion of carbapenem-hydrolyzing β-lactamases. Carbapenemases are enzymes that can hydrolyze most β-lactam rings including carbapenems, thus conferring resistance to these drugs [15].

As a result, infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae* (CPE) have become a major global concern due to their association with high (> 30%) case-fatality rates [16]. These enzymes have increasingly been reported as major health problems worldwide [17], particularly *Klebsiella pneumoniae* [18]. The most important concern regarding CPE is their high ability to cause outbreaks in healthcare settings [19]. The most predominant enzymes are New Delhi metallo-ß-lactamase (NDM) and OXA-48, which are mainly present around Mediterranean countries, it's worldwide distribution includes Europe, for instance; France, Germany, and Spain, and countries of North Africa namely Tunisia and Morocco [20].

Non-lactose-fermenting Gram-negative bacilli have often been overlooked, and increasingly develop resistance to carbapenems. They have developed as a major threat to critically ill patients [21], often immunosuppressed patients with significant comorbidities. As intrinsic antibiotic resistance to multiple antibiotic classes is common with these organisms, carbapenems are frequently used for treatment, rendering limited therapeutic options available. Among non-fermenting Gram-negative bacteria (NF-GNB), the most clinically significant species *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Stenotrophomonas maltophilia* are known carbapenem resistant species. Recently, the CDC classified AMRB according to their virulence and resistance determinants: urgent threats, serious threats, concerning threats, and watching list pathogens. The "urgent threat" pathogens included carbapenem-resistant *Acinetobacter* and carbapenem-resistant *Enterobacteriaceae*, whereas serious threat pathogens were ESBL-producing *Enterobacteriaceae*, multi-drug-resistant *Pseudomonas aeruginosa* and others.

Worldwide, the emergence of resistant Gram-negative bacteria is a global health threat. WHO has declared that AMR is one of the top 10 global public health threats facing humanity. Till now, little information related to this problem is available in the African region. This study aims to describe the phenotypic and molecular characteristics of carbapenem-resistant Gram-negative bacilli isolates recovered from hospitalized patients and hospital environments of three different medical settings in Libya.

Methodology

Collection history

This study was conducted at three hospitals located in different regions in Libya; Hospital 1 (Misurata

Medical Center (H1)), in Misurata, and swabs were collected from October to December 2018. Hospital 2 (Al-Jalaa Trauma Hospital) and Hospital 3 (Benghazi Medical Center (BMC)), H2 and H3, respectively. H2 and H3 hospitals are in Benghazi. Swabs were collected from October to November and from September to October 2019 for H2 and H3 respectively. H1 and H2 have 400 hundred beds each, whereas H3 is a tertiary hospital with 1200 beds. These hospitals have been chosen due to the frequency of fatal healthcare-associated infections in patients admitted to these hospitals.

A total of 379 samples were collected from these hospitals from patients, healthcare workers, the hospital environment, and sewage. Samples were as follows: 160 samples from H1 (24 samples from patients, 56 from healthcare workers, and 80 from hospital environment).

Bacterial isolates, data information, and identification

Three hundred seventy-nine (379) swabs were collected from hospitalized patients, medical staff, and the hospital environment. The swabs collected from patients were from different sites (Urine, blood, wound, hydrocephaly/shunt, and diabetic foot) from several departments (pediatric, neonatal ICU, male surgery ward, burn surgery shock room, intensive care unit (ICU), neurosurgery room, and neurosurgery shock room). The swabs collected from the hospital environment were from various surfaces (dressing room, bathrooms, scrub area, nursing room, suction machines, inside the baby incubator, bed mattress, and floor) from different wards (ICU, neonatal ICU, paediatric orthopedic ward, chest surgery room, burns and plastic surgery ward, and emergency theatre room).

All swabs collected from the three hospitals were grown on Brain Heart Infusion at 37 °C for 8 hours and then cultured on MacConkey agar supplemented with 2 mg/L of cefotaxime and incubated at 37°C for 18 – 24 hrs to select for resistant isolates. Isolates grown on MacConkey agar were then identified by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS).

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were performed using the standard disc diffusion method on Mueller–Hinton agar, as recommended by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf).

For *Enterobacteriaceae*, a total of 16 antimicrobial agents were tested, including β-lactam antibiotics: Amoxicillin (AMX), β-lactam/β-lactamase inhibitor complexes, amoxicillin-clavulanic acid (AMC), monocyclic β-lactam, aztreonam (ATM), cephalosporins, cefepime (FEP), cephalothin (KF), and ceftriaxone (CRO). Carbapenems were imipenem (IMP) and ertapenem (ETP), aminoglycosides: gentamicin (CN) and amikacin (AK), and doxycycline (DO), fluoroquinolones: ciprofloxacin; (CIP), fosfomycin (FF), nitrofurantoin (F), folate metabolic pathway inhibitors; trimethoprim-sulfamethoxazole (SXT), and polymyxin E (colistin) (CT).

For non-*Enterobacteriaceae*, a range of 16 antimicrobial agents was used, including β-lactam antibiotics; ticarcillin (TIC), β-lactam/β-lactamase inhibitor complexes, ticarcillin/clavulanic acid (TCC), piperacillin/tazobactam (TPZ), monocyclic β-lactam aztreonam (ATM), cephalosporins, cefepime (FEP) and ceftazidime (CAZ), carbapenems, imipenem (IMP), and meropenem (MEM), aminoglycosides, gentamicin (CN), amikacin (AK), fluoroquinolones, ciprofloxacin (CIP), folate metabolic pathway inhibitors, trimethoprim-sulfamethoxazole (SXT), rifampicin (RA), fosfomycin (FF), tetracyclines, doxycycline (DO), polymyxin E and colistin (CT).

β-Carba test for Enterobacteriaceae-resistant strains

Carbapenemase production was detected by the Carba NP test. This test was used to confirm if resistance was due to enzymatic or non-enzymatic mechanisms.

Detection of carbapenemase-encoding genes

The quantification of carbapenemase-encoding genes (bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48}, and bla_{OXA-58}) was performed by quantitative Light Cycler® real-time PCR (Roche Diagnostics, Meylan, France), using specific primers and probes [22]

Detection of oprD porin loss in P. aeruginosa

The amino acid changes of the protein *OprD* in imipenem-resistant *P. aeruginosa* isolates were detected using specific PCR primers, as previously described [23].

Results

Identification of bacterial isolates

A total of 379 samples were recovered from different sources from various hospitals in Libya. Two hundred ten samples (55.40%) were able to grow on

culture media. For Misurata Medical Centre (MMC) (H1), 160 samples were obtained. 41 samples were Gram-negative (percentage). For Al-Jala Hospital (H2), 120 samples were collected, sixty-nine samples were Gram-negative bacteria. For Benghazi Medical Center (BMC) (H3), only 23 isolates of Gram-negative bacteria were recovered from 99 samples collected.

Overall, only carbapenem-resistant strains were included (n = 133) in this study. Sixty-four strains were isolated from patients, the remaining 69 strains were from hospital environments.

Identification of isolates by MALDI-TOF showed that various Enterobacteriaceae species were found among the isolates (n = 84) with dominance of *Klebsiella pneumoniae* (n = 29 from patients, n = 3 from medical staff and n = 33 from hospital environment), followed by *Citrobacter freundii* (n = 2 from patients, n = 1 from medical staff and n = 1 from hospital environment). The results are illustrated in Supplementary Tables 1 and 3.

For non-fermenting bacilli, the results of identification revealed different species among the isolates (n = 49) with a preponderance of *A. baumannii* from patients and hospital environment (n = 16 and n = 8) followed by *P. aeruginosa* (n = 8 and n = 7).

Strains isolated from patients were recovered from different sites (nasal cavity, hand swab, thigh wound swab, wound swab, and hydrocephaly/shunt specimens), and strains from the hospital environment were collected from various wards (pediatric ward, neonatal ICU, male surgery ward, burn and plastic surgery ward, and ICUs). Environmental swabs were from different sites (boiling machine, suction machine, knob of refrigerator, and dressing room). All results are described in Table 1 and Supplementary Tables 2 and 4.

Antimicrobial susceptibility profile

Enterobacteriaceae isolates from patients

Antimicrobial susceptibility results are summarized in Supplementary Tables 1, 2, 3, and 4. The results showed that the majority of Enterobacteriaceae strains isolated from hospitalized patients in the three hospitals were represented mainly by β-lactams resistant *K. pneumoniae* (n = 26), including 100% resistance to amoxicillin and amoxicillin-clavulanic acid (AMC) (n = 26), followed by piperacillin-tazobactam (TPZ) (97.03%). For cephalosporins; the resistance rate was

Table 1. Molecular characteristics of Enterobacteriaceae and none Enterobacteriaceae isolated from three Libyan hospitals.

Hospital	Strain	Source of collection	MBL	CHCD	MBL + CHCD	Carbapenem resistance due to mutation, insertion sequence or other mechanisms
H1, H2, H3	*K. pneumoniae* (n = 29)	Patients / Medical staff	NDM-1 (n = 12); VIM-1 (n = 1); NDM-1, VIM-1 (n = 2)	OXA-48 (n = 5)	NDM-1, OXA-48 (n = 8)	-
H1	*E. coli* (n = 2)	Patients	-	-	NDM-1, OXA-48 (n = 2)	-
H1, H2, H3	*C. freundii* (n = 3)	Patients / Medical staff	NDM-1 (n = 2)	OXA-48 (n = 1)	-	-
H2	*E. xiangfangensis* (n = 1)	Patients	NDM-1 (n = 1)		-	-
H1, H2, H3	*A. baumannii* (n = 16)	Patients	NDM-1 (n = 7)	OXA-23 (n = 6)	NDM-1, OXA-23 (n = 2)	OprD (substitution, deletion) (n = 1); OprD (St'op codon) (n = 1); oprD (IS*Pa*26) (n = 1)
H2, H3	*P. aeruginosa* (n = 8)	Patients	VIM-2 (n = 4)	-	-	-
H2	*P. oleovorans* (n = 1); *P. mendocina* (n = 1); *P. putida* (n = 1); *P. otitides* (n = 1)	Patients	VIM-2 (n = 4)	-	-	-
H1, H2, H3	*K. pneumoniae* (n = 33)	Environment	NDM-1 (n = 20)	-	NDM-1, OXA-48 (n = 8); NDM-1, OXA-48, VIM-1 (n = 1)	-
H1, H2	*E. cloacae* (n = 9)		NDM-1 (n = 7)	OXA-48 (n = 1)		KPC-2 (n = 1)
H1, H2	*E. coli* (n = 4)		NDM-1 (n = 1)	OXA-48 (n = 1)	NDM-1, OXA-48 (n = 2)	
H1	*C. freundii* (n = 1)		NDM-4 (n = 1	-	-	-
H2	*R. ornithinolytica* (n = 1)		NDM-1 (n = 1)	-	-	-
H1	*S. marcescens* (n = 1)		-	OXA-48 (n = 1)	-	-
H1, H2, H3	*A. baumannii* (n = 7)		NDM-1 (n = 1)	OXA-23 (n = 6)	-	-
H1, H2	*P. aeruginosa*		VIM-2 (n = 4)	-	-	OprD (substitution, insertion) (n = 1); OprD (substitution) (n = 1); OprD (stop codon 646 pb) (n = 1)
H3	*S. maltophila*		-	OXA-23 (n = 1)	-	-
H2	*C. aquatica*		NDM-1 (n = 1)	-	-	-

100% for cephalothin, cefepime, and ceftriaxone., 93.33% showed reduced susceptibility to imipenem and ertapenem. Three isolates of *K. pneumoniae* (n = 3) collected from medical staff displayed a high rate of resistance to the β-lactam family, mainly to carbapenem antibiotics (imipenem and ertapenem), they also exhibited resistance to non-β-lactam antibiotics; aminoglycosides (Amikacin (n = 2)), quinolones (Ciprofloxacin (n = 3)), and tetracycline (Doxycycline (n = 3)).

Two *C. freundii* were isolated from patients and one from medical staff, the results showed that they exhibited variable antibiotic resistance profile, mainly to carbapenem antibiotics with resistance to imipenem and ertapenem. Only two strains of *E. coli* and one strain of *E. xiangfangensis* were recovered and were resistant to the β-lactam family, including carbapenem antibiotics (imipenem and ertapenem), a. The isolates also displayed resistance to β-lactam antimicrobial agents; fosfomycin, ciprofloxacin, and sulfamethoxazole.

The antibiotic susceptibility profile was further confirmed using E-test for imipenem and ertapenem. The MICs for imipenem and ertapenem were > 2 and > 0.5 ug/mL, respectively. The results presented in Supplementary Tables 1 and 3 showed that MICs of CR-Enterobacteriaceae ranged from > 2 to > 32 ug/mL for imipenem, while for ertapenem the level of resistance was between 0.5 to > 32 ug/mL.

The majority of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) were from ICU patients (n = 9), other patients were treated in general wards; surgery (n = 6), orthopedic (n = 6), pediatric (n = 2) and other different wards. CRKP were isolated from the nasal cavity of patients (n = 9), wound swabs (n = 7), diabetic foot (n = 3), armpit (n = 2), hydrocephaly (n = 1), and urine (n = 1). The results of antibiotic susceptibility testing for all isolated strains are shown in Supplementary Table 1.

Isolates from the hospital environment

Antibiotic susceptibility testing of *Enterobacteriaceae* isolates from the hospital environment is summarized in Supplementary Table 3. The results showed that *K. pneumoniae* (n = 33), were 100% resistant to amoxicillin, amoxicillin/ clavulanic acid, piperacillin/tazobactam and ceftriaxone, followed by cefepime and cephalothin (96.96%). *K. pneumoniae* isolates were 100% resistant to carbapenem antibiotics (ertapenem and imipenem). The remaining strains: *E. cloacae* (n = 9) displayed 100% resistance to imipenem and ertapenem, followed by *E. coli* (n = 4), *C. freundii* (n = 1), *M. ornithinolytica*, and *S. marcescens* (n = 1).

Non-Enterobacteriaceae

Concerning non-fermenting bacteria, clinical isolates were isolated from wounds (n = 7), nasal swabs (n = 3), Tip of Foley's catheters (n = 3), hydrocephaly/sore (n = 1), blood (n = 1), and urine (n = 1). These strains showed a high level of resistance against many antimicrobial agents. For *A. baumannii* isolates (n = 16), the results demonstrated a high level of resistance to β-lactams; ticarcillin, ticarcillin/clavulanate, cefepime, and ceftazidime (100%). They were 100% resistant to imipenem and meropenem (Supplementary Table 2).

Our findings showed that strains of *P. aeruginosa* (n = 8) exhibited 100% carbapenem resistance (imipenem and meropenem) for specimens recovered from patients. *P. mandocina*, *P. putida,* and *P. oleovorans* were resistant to carbapenems (imipenem and meropenem), they were isolated from the head wound and nasal swabs from patients in the neurosurgery shock room, burn surgery shock room and ICU wards. *P. otitides* was resistant to meropenem, it was recovered from a nasal swab from a patient in the burn surgery shock room.

The carbapenem resistance profile of all isolates was confirmed by determining the MIC value of imipenem by the E-test method (BioMérieux, Marcy-l'Etoile, France). All resistant strains exhibited MIC values of > 32 µg/mL for imipenem. In addition, > 90% *A. baumannii* and > 60%, *P. aeruginosa* isolates were resistant to aminoglycosides, macrolides, tetracycline, and cotrimoxazole. Polymyxin E (colistin) was the most effective.

The results of antimicrobial susceptibility testing of *Acinetobacter* and *Pseudomonas* spp. (Supplementary Table 4) isolated from the hospital environment showed that *A. baumannii* isolates (n = 8), had a high level of resistance (100%) to all β-lactam family drugs, including ticarcillin, ticarcillin/clavulanic acid, piperacillin/tazobactam, cephalosporins, carbapenem antibiotics (imipenem and meropenem). Furthermore. For *Pseudomonas* spp strains, *P. aeruginosa* (n = 7), *P. putida* (n = 1), *P. otitidis* (n = 1), were 100% resistant to carbapenems. The carbapenem resistance profile of all isolates was confirmed by measuring the MIC value of imipenem using the E-test method (BioMérieux, Marcy-l'Etoile, France). Almost all resistant strains exhibited MIC value > 32 µg/mL.

Carba NP test results

In this study, four strains of *Enterobacteriaceae* (*K.*

pneumoniae) with reduced susceptibility to ertapenem and intermediate to imipenem, recovered from clinical and environmental settings, were unable to express studied carbapenemase genes. Test results showed that strains remained yellow, which might explain that resistance was due to a non-enzymatic mechanism.

Molecular characterization of carbapenemase-encoding genes

Enterobactereales

A total of 84 Enterobacteriaceae strains (n = 31 clinical isolates, n = 49 hospital environmental isolates, and n = 4 from medical staff) were screened for carbapenemase-encoding genes by PCR and subsequent sequencing. The isolates yielded positive PCR products for carbapenemase-encoding genes: *bla*$_{KPC}$, *bla*$_{OXA-23}$, *bla*$_{OXA-48}$, and *bla*$_{NDM}$. All molecular findings are illustrated in Table 1 and Supplementary Tables 1 and 3.

bla$_{NDM}$ was the most prevalent carbapenemase detected among CRKP strains in 22 and 29 of the clinical and hospital environmental samples, respectively. Clinical strains were from nasal swabs (n = 9), wounds (n = 8), armpits (n = 2); and diabetic foot (n = 3), less extent, from hydrocephaly/shunts, and tips of Foley's catheter (n = 1), hospital environmental strains were mainly from bathrooms (n = 10), suction machine (n = 3), floor surface (n = 3), sewage (n = 3), from different wards, children orthopaedic ward, male orthopaedic ward, male surgery ward, and neurosurgery shock room.

Furthermore, *bla*$_{NDM}$ was detected in *E. coli* (n = 2, n = 3), in *C. freundii* (n = 2, n = 1) from clinical and hospital environmental samples, respectively, and from seven *E. cloacae* strains recovered from the hospital environment. To a lesser extent, this enzyme was detected in *E. xiangfangensis* and *R. ornithinolytica* isolated from Foley's catheter from a patient admitted to the surgery shock room, and from the bathroom in the female surgery ward.

Investigation of class D oxacillinase among our CRKP strains showed that *bla*$_{OXA-48}$ was the most prevalent enzyme in 12 clinical strains recovered from various sites, mainly from thigh and foot wounds (n = 6), nasal swabs (n = 3) and other sites. OXA-48 enzyme was identified in *E. coli* (n = 2), and *C. freundii* (n = 1) isolated from the armpit and nasal cavity of patients in the ICU ward. It was surprising that a hand swab of medical staff was positive for *bla*$_{OXA-48}$.

Furthermore, *bla*$_{OXA-48}$ was detected in the hospital environment from sewage, suction machine, floor, and refrigerator knob, in *K. pneumoniae* (n = 10), *E. coli* (n = 3), *E. cloacae* (n = 1) and *S. marcescens* (n = 1). Ertapenem MICs for all isolates with OXA-48 varied from (0.5 –32 ug/mL), and MIC*s* for imipenem ranged from 0.38 - > 32 ug/mL. Ertapenem is known to be mostly affected by OXA-48 carbapenemase. *Bla*$_{KPC}$ was detected only in one strain of *E. cloacae* isolated from the floor of the ICU, Misurata Medical Center.

While *bla*$_{VIM}$ was detected in two *K. pneumoniae*, these strains were recovered from the armpit of the patient admitted to ICU and from the hand swab of medical staff from the same ICU.

Non-Enterobacteriaceae

A total of 49 non-Enterobacteriaceae strains (n = 28 from clinical isolates, n = 20 from hospital environment, and n = 1 from medical staff) were screened for carbapenemase-encoding genes by PCR and subsequent sequencing. The isolates yielded positive PCR for carbapenemase-encoding genes; *bla*$_{OXA-23}$, *bla*$_{VIM}$, and *bla*$_{NDM}$ for strains isolated from clinical samples and hospital environments. All molecular findings are illustrated in Table 1 and Supplementary Tables 2 and 4.

A. baumannii

PCR results showed that all CR-*A. baumannii* isolates harbored at least one of the genes under investigation; 14 strains (58.33%) were positive for *bla*$_{OXA-23}$ (n = 8 from clinical samples, n = 6 from hospital environment swabs). In addition, 10 isolates (40.9%) tested positive for the metallo β-lactamase *bla*$_{NDM}$, in which (n = 9) were from patients (Tip of Foley's catheter, hydrocephaly/sore, wounds, and nasal swabs) and only one strain was from inside baby incubator in neonatal ICU. In this study, the isolates were negative for *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{OXA-24}$, and *bla*$_{OXA-58}$. Additionally, two strains co-harbored *bla*$_{OXA23}$ and *bla*$_{NDM}$ were from the tip of the endotracheal tube, and wound in the pediatric ward, of the same hospital. E-test showed that all meropenem and imipenem MIC values were ≥ 32 µg/mL (i.e., the maximum value on *the* meropenem strip). All these isolates were colistin-sensitive by disc diffusion, in contrast, colistin MIC values ranged from 0.5 - to 2 µg/mL.

Pseudomonas spp.

CR-*P. aeruginosa* isolates harbored at least one of the tested genes, and 8 isolates tested negative for the studied genes. The other eight CR-*P. aeruginosa* strains were positive by PCR for *bla*$_{VIM}$. Four isolates (n = 4) were from clinical samples, and the others (n = 4), were

swabbed from environmental settings (bathrooms and suction machines) in neurosurgery shock room and ICU wards. No other carbapenemase genes were detected.

E-test showed that all imipenem, and meropenem MIC values were ≥ 32 µg/mL. All strains were sensitive to colistin, the MIC ranged from 0.5 to 2 µg/mL. Furthermore, carbapenemase genes were investigated in species other than *P. aeruginosa*, VIM-2 gene was detected in *P. putida* (n = 1; n = 1) from one patient and hospital environment, *P. otitidis* (n = 1), *P. oleovorans* (n = 1), *P. mandocina* (n = 1) harvested from, burn and plastic, burn surgery shock room, and ICU wards.

For strains of *Pseudomonas* resistant to imipenem with no carbapenemase-producing genes, PCR, and sequencing of oprD gene were conducted. Insertion sequence *IS*Pa26 was detected in one strain from a urine sample of a 67-year-old male. Modification of OprD with substitution/deletion was detected in three strains, and stop codon was detected in one strain.

Discussion

The occurrence and dissemination of antimicrobial-resistant bacteria (AMR) responsible for severe infections is currently a major concern. Infections caused by carbapenemase-producing *Enterobacteriaceae* (CPE) lead to increased mortality [24]. CPE infections are constantly increasing worldwide and spreading to several countries.

In our study, bla_{NDM} was the most prevalent enzyme produced by CR-*Enterobacteriaceae* (n = 84). NDM was first detected in 2008 [25] from *K. pneumoniae,* and one year later from *E. coli* [26]. NDM-1 appeared to be endemic in the Indian subcontinent [27]. Since then, bla_{NDM-1} *Enterobacteriaceae* have been identified and reported all over the world, Mediterranean area; Tunisia [28], Morocco [29], and Egypt [24]. NDM was mostly from CRKP where 49 strains displayed this enzyme. Our findings suggest that the North African region could be a significant source of propagation and dissemination of bla_{NDM} isolates, due to a high movement between Libya and neighbour countries. Most CRKPs were isolated from ICUs, which is in line with other findings [24,28]. Moreover, *E. cloacae* became of clinical importance as an opportunistic bacteria and emerged as a nosocomial pathogen in ICU patients on mechanical ventilation [29]. To the best of our knowledge, this is the first report of the occurrence of NDM-1-producing *C. freundii* in Libya.

Moreover, a total of 41 NDM-producing strains were detected in the hospital environment, a high incidence by CRKP (n = 29). The remaining species were *E. cloacae* (n = 7), *E. coli* (n = 3), *C. freundii* (n = 1), and *R. ornithinolytica* (n = 1). The majority of bla_{NDM} was identified from sewage (n = 15) from various surgical, orthopaedic, and ICUs. Our results are in line with previous findings, suggesting that hospital wastewater is an important source of NDM-1-producing bacteria.

OXA-48 is an emerging resistance mechanism in *Enterobacteriaceae*, which may complicate the problem of HAIs and hospital contamination [30]. Our results showed that bla_{OXA-48} (n = 16), found in the three hospital settings, was mainly reported from *K. pneumoniae* (n = 12), *E. coli* (n = 2), and *C. freundii* isolated from medical staff. These strains were mostly from nasal cavity (n = 4), wounds, and diabetic foot swabs (n = 3). This is the second study of the emergence of OXA-48 in Libyan hospitals. The first study was performed by Mathlouthi *et al.*, in 2016, in which seven *K. pneumoniae* isolated from tracheal swabs from patients admitted to the ICU in Libya exhibited bla_{OXA-48} [31]. Nevertheless, this is the first finding describing the occurrence of bla_{OXA-48} in a *C. freundii* strain.

The most significant occurrence of OXA-48-producing isolates of *K. pneumoniae* in the Mediterranean basin was reported from Italy [32]. The occurrence of OXA-48-producing isolates has been reported in many countries in the Mediterranean area, including Greece, Lebanon, Egypt, Libya, Tunisia, Algeria, and Morocco [33].

In this study, Bla_{OXA-48} was detected in the hospital environment, in *K. pneumoniae* (n = 9), *E. coli* (n = 3), *E. cloacae* (n = 1), *C. freundii* (n = 1), and *S. marcescens* (n = 1). To the best of our knowledge, this is the first time bla_{OXA-48} and bla_{NDM} genes have been detected from bathrooms in hospitals, mainly in CRKP in Libya.

In Tunisia, a neighbour country, a study conducted by Nasri *et al.* in 2017 highlighted the emergence of bla_{OXA-48} in effluent water from seven hospitals, which reinforces our findings [34]. North Africa has been considered one of the main reservoirs of OXA-48-producing isolates [35]. bla_{KPC} was detected only in one strain of *E. cloacae* harvested from the floor of the ICU. In 1996 KPC producers were reported from in the United States, and Mediterranean countries, particularly Italy, Greece, and Israel. They are now endemic for the bla_{KPC} gene [36].

In this work, for clinical strains, bla_{OXA-48} was associated with bla_{NDM} in eight *K. pneumoniae* strains, mainly from wounds (n = 3), nasal cavity (n = 1), diabetic foot (n = 2), hydrocephaly/shunt (n = 1) and baby hand (n = 1), most of them were from surgery and ICU wards. Several studies reported the occurrence of

more than one carbapenemase-encoding gene in the same isolate [28]. Such association was described in various studies, including Italy [37], Brazil [38], and North Africa [39].

Concerning hospital environments, *bla*$_{NDM}$ was co-expressed with *bla*$_{OXA-48}$ in eight strains of *K. pneumoniae* and two strains of *E. coli* from various sites, mainly sewage from different wards (ICU, neonatal ICU, and surgery ward). It is worth mentioning that *bla*$_{NDM}$ was combined with *bla*$_{OXA-48}$ and *bla*$_{VIM}$ in one strain of *K. pneumoniae* harvested from sewage in the neonatal ICU. Such co-occurrence was detected in several studies in different areas, demonstrating that hospitals can be a reservoir of CRE, harbouring clinically relevant carbapenemases [40].

MDR *A. baumannii* outbreaks were reported in hospitals worldwide, including the Middle East [41] and North Africa [42]. OXA-type β-lactamases played a role in the emergence of CRAB isolates and are the most prevalent carbapenemases in *A. baumannii* [43]. Production of *bla*$_{OXA-23}$ by an *A. baumannii* strain is enough to confer resistance to carbapenems. In this study, carbapenem resistance was mainly attributed to the carriage of OXA-23 gene that was present in most clinical isolates (n = 8). It was recovered from various sites; wound (n = 2), nasal cavity (n = 2), Tip of Foley's catheter (n = 2), Tip of endotracheal tube (n = 1), hydrocephaly/sores (n = 1), from ICU and surgical wards. This gene is plasmid-borne, suggesting that the mobility of this genetic element facilitates horizontal gene transfer [44]. This finding reinforces a previous study performed by Mathlouthi *et al.* (2016) that highlighted the occurrence of *bla*$_{OXA-23}$ in various sites and wards [45].

About 56.25% (n = 9) of our *A. baumannii* isolates tested positive for class B β-carbapenemase (*bla*$_{NDM-1}$). CP-AB were recovered from different sites (blood, diabetic foot, wound, and endotracheal tube), mainly from the ICU. This is in line with the finding of Mathlouthi *et al.*, 2016, where strains of *A. baumannii* harbouring *bla*$_{NDM}$ recovered from different sites. Identification of several clinical *A. baumannii* isolates harbouring *bla*$_{NDM-1}$ gene and originating from North Africa, with no obvious link to the Indian subcontinent, strongly suggests that NDM-1-producing *A. baumannii* clones probably widespread in North Africa and might act as a reservoir for *bla*$_{NDM-1}$.These results are consistent with regional and global findings; reports from Israel, India, China, United Kingdom, Canada, France, Sweden, Morocco, South Africa, United Arab Emirates, and Iran [46].

Regarding the hospital environment, data showed that *bla*$_{OXA-23}$ was detected in six *A. baumannii* from suction machines, bathrooms, and mechanical ventilators. *bla*$_{NDM}$ was detected in only one isolate of *A. baumannii* from inside a baby incubator in the neonatal ICU. Our results are consistent with the recent finding by Bonin *et al.* (2019) in a French hospital, which highlighted the occurrence mainly of OXA-23 and a lesser extent of NDM in carbapenem-resistant *A. baumannii* in a hospital environment [47]. Our findings are lower than a study performed by Zenati *et al.*, 2016 in Algeria, which described *bla*$_{OXA-23}$ and *bla*$_{NDM-1}$ genes in 29 and 32 strains of carbapenem-resistant *A. baumannii* isolates, respectively [48].

P. aeruginosa is an environmental species and one of the most frequent nosocomial pathogens [49]. Its ability to develop multi-drug resistance renders infections very difficult to treat resulting in a high mortality rate, ranging from 18% to 61% [50]. Carbapenems (including imipenem, meropenem, and doripenem) are often used as the drug of choice against multidrug-resistant *P. aeruginosa* for the treatment of serious infections such as nosocomial pneumonia, serious nosocomial intra-abdominal infection, and septicemia caused by this pathogen. In the present work, a total of 15 *P. aeruginosa* recovered from clinical specimens (n = 8) and various hospital environment swabs (n = 7), from three medical settings in Libya were studied. Most clinical strains harboured VIM-2 carbapenemase-encoding gene (n = 4), mainly from wounds. *bla*$_{VIM}$ was detected in four strains of CRPA harvested from suction machines and bathrooms. These findings showed a higher occurrence of *bla*$_{VIM}$ than the findings of Maroui *et al.* (2016) in Morocco and Safraoui *et al.* (2014) in Algeria, in which only two strains displayed VIM-2 were detected [51,52].

Carbapenem resistance in *P. aeruginosa* strains may result from multiple mechanisms with or without the production of carbapenemases. Permeability changes in the outer membrane and the occurrence of protein loss (oprD), play a crucial role in resistance to carbapenem antibiotics in *P. aeruginosa*. Our results showed that CRPA displayed a modified OprD (n = 3 from clinical isolates, n = 3 from hospital environment), in which one strain with an IS by sequencing and Blast with PAO1 gene showed identity with IS*Pa*26, and the other strains harboured a polymorphism (stop codon, insertion, deletion, and substitution). Our results are in line with many findings all over the world, highlighting that an IS in the oprD gene plays a role in the resistance of *P. aeruginosa* to carbapenems [53].

Conclusions

In the current study, it can be confirmed that the hospital environment including toilet, sink, and drain removal, provides a rich niche for propagation of bacteria and especially a source of carbapenem-producing bacteria. Inside the hospital, water access and drainage systems could facilitate MDRB transmission through direct or indirect contact with water, or from droplets produced during water-related activities, underlining the importance of water distribution and wastewater design to reduce such risk. Ensuring effective infection prevention and control (IPC) measures is important to eradicate reservoirs of pathogens and prevent MDRB cross-transmission between patients, healthcare workers, and the environment, which may minimize potentially lethal outbreaks, knowing the predominant site for each organism may help guide surveillance strategies.

Authors' Contributions

Conceptualization, data curation, and formal analysis: Khouloud Slimene, Salem K. Almahjoub, Elhussan A. Mohamed. Investigation and methodology: Khouloud Slimene, Aldukali Abdulsalam Alkeskas, Mohammed, I. Hameid, Souad Alsanosi, Ahmed I. Elbousify. Supervision and validation: Allaaeddin El Salabi, Jean-Marc Rolain, Chedly Chouchani. Writing original draft: Khouloud Slimene, Allaaeddin El. Salabi. Writing – review and editing: Elham O. Omar, Allaaeddin El Salabi, Jean-Marc Rolain, Chedly Chouchani.

Funding

This work was funded partly by IHU Méditerranée Infection, Aix-Marseille-University in France. - This work was supported by the Tunisian Ministry of Higher Education and Scientific Research and Campus France Under the PHC-Utique project (Code 18G0819), which offered a scholarship to Miss. Khouloud Slimene to attend the IHU Méditerranée Infection, Aix-Marseille-University in France.

Corresponding author

Professor Chedly Chouchani
Université de Carthage, ISSTE de Borj-Cedria,
BP-1003, Hammam-Lif 2050,
Tunisie
Email: chedly.chouchani@isste.ucar.tn

Conflict of interests

No conflict of interests is declared.

References

1. Dancer SJ (2009) The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect 73: 378–385. doi: 10.1016/j.jhin.2009.03.030.
2. Wagenvoort JHT, Penders RJR (1997) Long-term in-vitro survival of an epidemic MRSA phage-group III-29 strain. J Hosp Infect 35: 322–325. doi: 10.1016/S0195-6701(97)90265-0.
3. Rüden H, Gastmeier P, Daschner FD, Schumacher M (1997) Nosocomial and community-acquired infections in Germany. Summary of the results of the first national prevalence study (NIDEP). Infection 25: 199–202. doi: 10.1007/BF01740822.
4. Cohen B, Cohen CC, Løyland B, Larson E (2017) Transmission of health care-associated infections from roommates and prior room occupants: a systematic review. Clin Epidemiol 9: 297–310. doi: 10.2147/CLEP.S130634.
5. Hirai Y (1991) Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. J Hosp Infect 19: 191–200. doi: 10.1016/0195-6701(91)90213-3.
6. Hocquet D, Muller A, Bertrand X (2016) What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J Hosp Infect 93: 395–402. doi: 10.1016/j.jhin.2016.01.010.
7. Seale AC, Gordon NC, Islam J, Peacock SJ, Scott JAG (2017) AMR Surveillance in low and middle-income settings - A roadmap for participation in the Global Antimicrobial Surveillance System (GLASS). Wellcome Open Res 2: 92. doi: 10.12688/wellcomeopenres.12527.1.
8. Kalaiselvi K, Mangayarkarasi V, Balakrishnan D, Chitraleka V (2016) Survival of antibacterial resistance microbes in hospital-generated recycled wastewater. J Water Health 14: 942–949. doi: 10.2166/wh.2016.046.
9. Lien TQ, Lan PT, Chuc NTK, Hoa NQ, Nhung PH, Thoa NTM, Diwan V, Tamhankar AJ, Stålsby Lundborg C (2017) Antibiotic resistance and antibiotic resistance genes in *Escherichia coli* isolates from hospital wastewater in Vietnam. Int J Environ Res Public Health 14: 699. doi: 10.3390/ijerph14070699.
10. Mills G (2015) Living better in the built environment: make sure the environment of care is both safe and comfortable for patients, visitors, and staff. Jt Comm Perspect 35: 9–11.
11. Rock C, Anderson M, Lewis S, Scheeler V, Nowakowski E, Hsu YJ, Milstone AM, Carroll KC, Maragakis LL, Simner PJ; CDC Prevention Epicenters Program (2018) Comparison of nylon-flocked swab and cellulose sponge methods for carbapenem-resistant *Enterobacteriaceae* and gram-negative organism recovery from high-touch surfaces in patient rooms. Infect Control Hosp Epidemiol 39: 1257–1261. doi: 10.1017/ice.2018.174.
12. Marchaim D, Gottesman T, Schwartz O, Korem M, Maor Y, Rahav G, Karplus R, Lazarovitch T, Braun E, Sprecher H, Lachish T, Wiener-Well Y, Alon D, Chowers M, Ciobotaro P, Bardenstein R, Paz A, Potasman I, Giladi M, Schechner V, Schwaber MJ, Klarfeld-Lidji S, Carmeli Y (2010) National multicenter study of predictors and outcomes of bacteremia upon hospital admission caused by *Enterobacteriaceae* producing extended-spectrum beta-lactamases. Antimicrob Agents Chemother 54: 5099–5104. doi: 10.1128/AAC.00756-10.
13. Karah N, Sundsfjord A, Towner K, Samuelsen Ø (2012) Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. Drug Resist Updat 15: 237–247. doi: 10.1016/j.drup.2012.06.001.
14. Jesudason MV, Kandathil AJ, Balaji V (2005) Comparison of two methods to detect carbapenemase & metallo-beta-lactamase production in clinical isolates. Indian J Med Res 121: 780–783. doi: 10.4103/0971-5916.16818.

15. Wu W, Espedido B, Feng Y, Zong Z (2016) *Citrobacter freundii* carrying blaKPC-2 and blaNDM-1: characterization by whole genome sequencing. Sci Rep 6: 30670. doi: 10.1038/srep30670.
16. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M (2012) Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. Clin Infect Dis 55: 943–950. doi: 10.1093/cid/cis581.
17. Navarro-San Francisco C, Mora-Rillo M, Romero-Gómez MP, Moreno-Ramos F, Rico-Nieto A, Ruiz-Carrascoso G, Gómez-Gil R, Arribas-López JR, Mingorance J, Paño-Pardo JR (2013) Bacteraemia due to OXA-48-carbapenemase-producing *Enterobacteriaceae*: a major clinical challenge. Clin Microbiol Infect 19: E72–E79. doi: 10.1111/1469-0691.12075.
18. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH (2016) Global dissemination of carbapenemase-producing *klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol 7: 895. doi: 10.3389/fmicb.2016.00895.
19. French CE, Coope C, Conway L, Higgins JPT, McCulloch J, Okoli G, Patel BC, Oliver I (2017) Control of carbapenemase-producing *Enterobacteriaceae* outbreaks in acute settings: an evidence review. J Hosp Infect 95: 3–45. doi: 10.1016/j.jhin.2016.11.012.
20. Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A (2018) OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. Eur J Clin Microbiol Infect Dis 37: 587–604. doi: 10.1007/s10096-017-3144-4.
21. Kim UJ, Kim HK, An JH, Cho SK, Park KH, Jang HC (2014) Update on the epidemiology, treatment, and outcomes of carbapenem-resistant *Acinetobacter* infections. Chonnam Med J 50: 37–44. doi: 10.4068/cmj.2014.50.2.37.
22. Mesli E, Berrazeg M, Drissi M, Bekkhoucha SN, Rolain JM (2013) Prevalence of carbapenemase-encoding genes including New Delhi metallo-β-lactamase in *Acinetobacter* species, Algeria. Int J Infect Dis 17: e739–e743. doi: 10.1016/j.ijid.2013.03.011.
23. Wolter DJ, Hanson ND, Lister PD (2004) Insertional inactivation of oprD in clinical isolates of *Pseudomonas aeruginosa* leading to carbapenem resistance. FEMS Microbiol Lett 236: 137–143. doi: 10.1111/j.1574-6968.2004.tb09655.x.
24. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ (2014) Deaths attributable to carbapenem-resistant *Enterobacteriaceae* infections. Emerg Infect Dis 20: 1170–1175. doi: 10.3201/eid2007.140545.
25. Huang X, Cheng X, Sun P, Tang C, Ni F, Liu G (2018) Characteristics of NDM-1-producing *Klebsiella pneumoniae* ST234 and ST1412 isolates spread in a neonatal unit. BMC Microbiol 18: 186. doi: 10.1186/s12866-018-1305-9.
26. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (2009) Characterization of a new metallo-beta-lactamase gene, bla (NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 53: 5046–5054. doi: 10.1128/AAC.00774-09.
27. Dortet L, Poirel L, Nordmann P (2014) Worldwide dissemination of the blaNDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int 2014: 249856. doi: 10.1155/2014/249856.
28. Ben Helal R, Dziri R, Chedly M, Klibi N, Barguellil F, El Asli MS, Moussa MB (2018) Occurrence and characterization of carbapenemase-producing *Enterobacteriaceae* in a Tunisian Hospital. Microb Drug Resist 24: 1361–1367. doi: 10.1089/mdr.2017.0336.
29. Barguigua A, Zerouali K, Katfy K, El Otmani F, Timinouni M, Elmdaghri N (2015) Occurrence of OXA-48 and NDM-1 carbapenemase-producing *Klebsiella pneumoniae* in a Moroccan university hospital in Casablanca, Morocco. Infect Genet Evol 31: 142–148. doi: 10.1016/j.meegid.2014.11.024.
30. Yousfi H, Hadjadj L, Dandachi I, Lalaoui R, Merah A, Amoura K, Dahi A, Dekhil M, Messalhi N, Diene SM, Baron S, Rolain JM (2019) Colistin-and carbapenem-resistant *Klebsiella pneumoniae* clinical isolates: Algeria. Microb Drug Resist 25: 258–263. doi: 10.1089/mdr.2018.0114.
31. Mathlouthi N, Al-Bayssari C, El Salabi A, Bakour S, Ben Gwierif S, Zorgani AA, Jridi Y, Ben Slama K, Rolain JM, Chouchani C (2019) Carbapenemases and extended-spectrum β-lactamases producing *Enterobacteriaceae* isolated from Tunisian and Libyan hospitals. J Infect Dev Ctries 10: 718–727. doi: 10.3855/jidc.11351.
32. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, Cantón R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Monnet DL, European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group (2017) Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE): a prospective, multinational study. Lancet Infect Dis 17: 153–163. doi: 10.1016/S1473-3099(16)30257-2.
33. Aggoune N, Tali Maamar H, Assaous F, Guettou B, Laliam R, Benamrouche N, Zerouki A, Naim M, Rahal K (2018) Wide spread of OXA-48-producing *Enterobacteriaceae* in Algerian hospitals: A four years' study. J Infect Dev Ctries 12: 1039–1044. doi: 10.3855/jidc.10441.
34. Nasri E, Subirats J, Sànchez-Melsió A, Mansour HB, Borrego CM, Balcázar JL (2017) Abundance of carbapenemase genes (blaKPC, blaNDM and blaOXA-48) in wastewater effluents from Tunisian hospitals. Environ Pollut 229: 371–374. doi: 10.1016/j.envpol.2017.05.064.
35. Nordmann P, Poirel L (2014) The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. Clin Microbiol Infect 20: 821–830. doi: 10.1111/1469-0691.12719.
36. van Duin D, Doi Y (2017) The global epidemiology of carbapenemase-producing *Enterobacteriaceae.* Virulence 8: 460–469. doi: 10.1080/21505594.2016.1231293
37. Gona F, Bongiorno D, Aprile A, Corazza E, Pasqua B, Scuderi MG, Chiacchiaretta M, Cirillo DM, Stefani S, Mezzatesta ML (2018) Emergence of two novel sequence types (3366 and 3367) NDM-1- and OXA-48-co-producing *K. pneumoniae* in Italy. Eur J Clin Microbiol Infect Dis 38(9): 1687–1691. doi: 10.1007/s10096-018-3327-4.
38. Flores C, Bianco K, de Filippis I, Clementino MM, Romão CMCPA (2019) Genetic relatedness of NDM-Producing *Klebsiella pneumoniae* co-occurring VIM, KPC, and OXA-48 enzymes from surveillance cultures from an intensive care unit. Microb Drug Resist 26: 1219–1226. doi: 10.1089/mdr.2019.0207.

39. Kraiem AG, Zorgani A, Elahmer O, Hammami A, Chaaben BM, Ghenghesh KS (2015) New Delhi metallo-β-lactamase and OXA-48 carbapenemases in Gram-negative Bacilli isolates in Libya. Libyan J Med 10. doi: 10.3402/ljm.v10.28714.
40. Zurfluh K, Bagutti C, Brodmann P, Alt M, Schulze J, Fanning S, Stephan R, Nüesch-Inderbinen M (2017) Wastewater is a reservoir for clinically relevant carbapenemase- and 16s rRNA methylase-producing *Enterobacteriaceae*. Int J Antimicrob Agents 50: 436–440. doi: 10.1016/j.ijantimicag.2017.04.015.
41. Henig O, Weber G, Hoshen MB, Paul M, German L, Neuberger A, Gluzman I, Berlin A, Shapira C, Balicer RD (2015) Risk factors for and impact of carbapenem-resistant *Acinetobacter baumannii* colonization and infection: matched case–control study. Eur J Clin Microbiol Infect Dis 34: 2063–2068. doi: 10.1007/s10096-015-2479-4.
42. Khorsi K, Messai Y, Hamidi M, Ammari H, Bakour R (2015) High prevalence of multidrug-resistance in *Acinetobacter baumannii* and dissemination of carbapenemase-encoding genes blaOXA-23-like, blaOXA-24-like and blaNDM-1 in Algiers hospitals. Asian Pac J Trop Med 8: 438–446. doi: 10.1016/j.apjtm.2015.05.011.
43. da Silva KE, Maciel WG, Croda J, Cayô R, Ramos AC, de Sales RO, Kurihara MNL, Vasconcelos NG, Gales AC, Simionatto S (2018) A high mortality rate associated with multidrug-resistant *Acinetobacter baumannii* ST79 and ST25 carrying OXA-23 in a Brazilian intensive care unit. PLoS One 13: e0209367. doi: 10.1371/journal.pone.0209367.
44. Mugnier PD, Poirel L, Naas T, Nordmann P (2010) Worldwide dissemination of the blaOXA-23 Carbapenemase gene of *Acinetobacter baumannii*. Emerg Infect Dis 16: 35. doi: 10.3201/eid1601.091225.
45. Mathlouthi N, El Salabi AA, Ben Jomàa-Jemili M, Bakour S, Al-Bayssari C, Zorgani AA, Kraiema A, Elahmer O, Okdah L, Rolain JM, Chouchani C (2016) Early detection of metallo-β-lactamase NDM-1-and OXA-23 carbapenemase-producing *Acinetobacter baumannii* in Libyan hospitals. Int J Antimicrob Agents 48: 46–50. doi: 10.1016/j.ijantimicag.2016.03.019.
46. Berrazeg M, Diene S, Medjahed L, Parola P, Drissi M, Raoult D, Rolain J (2014) New Delhi Metallo-beta-lactamase around the world: an eReview using Google Maps. Euro Surveill 19: 20809. doi: 10.2807/1560-7917.ES2014.19.20.20809.
47. Bonnin RA, Levy M, Cuzon G, Dortet L, Naas T (2017) Carbapenemase-producing *Acinetobacter spp.* from environmental sources in a hospital in French Polynesia. J Glob Antimicrob Resist 16: 81–82. doi: 10.1016/j.jgar.2017.09.004.
48. Zenati K, Touati A, Bakour S, Sahli F, Rolain JM (2017) Characterization of NDM-1- and OXA-23-producing *Acinetobacter baumannii* isolates from inanimate surfaces in a hospital environment in Algeria. J Hosp Infect 92: 19–26. doi: 10.1016/j.jhin.2015.09.009.
49. Borgatta B, Lagunes L, Imbiscuso AT, Larrosa MN, Lujàn M, Rello J (2017) Infections in intensive care unit adult patients harboring multidrug-resistant *Pseudomonas aeruginosa*: implications for prevention and therapy. Eur J Clin Microbiol Infect Dis 36: 1097–1104. doi: 10.1007/s10096-017-2915-9.
50. Poole K (2011) *Pseudomonas aeruginosa*: resistance to the max. Front Microbiol 2: 65. doi: 10.3389/fmicb.2011.00065.
51. Maroui I, Barguigua A, Aboulkacem A, Ouarrak K, Sbiti M, Louzi H, Timinouni M, Belhaj A (2016) First report of VIM-2 metallo-β-lactamases producing *Pseudomonas aeruginosa* isolates in Morocco. J Infect Chemother 22: 127–132. doi: 10.1016/j.jiac.2015.11.005.
52. Sefraoui I, Berrazeg M, Drissi M, Rolain JM (2014) Molecular epidemiology of carbapenem-resistant *Pseudomonas aeruginosa* clinical strains isolated from western Algeria between 2009 and 2012. Microb Drug Resist 20: 156–161. doi: 10.1089/mdr.2013.0128.
53. Kao CY, Chen SS, Hung KH, Wu HM, Hsueh PR, Yan JJ, Wu JJ (2016) Overproduction of active efflux pump and variations of OprD dominate in imipenem-resistant *Pseudomonas aeruginosa* isolated from patients with bloodstream infections in Taiwan. BMC Microbiol 16: 107. doi: 10.1186/s12866-016-0725-4.

Annex – Supplementary Items

Supplementary Table 1. Phenotypic and genotypic features of *Enterobacteriaceae* strains isolated from patients and medical staff of three Libyan hospitals.

Strain	ID	Age	Gender	Site	Ward	Hospital	Antibiotic susceptibility profile	E-Test IMP (µg/mL)	E-Test ETP (µg/mL)	Carbapenemase-producing gene
138 NF1	*K. pneumoniae*	75Y	F	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, CN, AK, F, CIP, DO	> 32	24	NDM-1
33 T	*K. pneumoniae*	61Y	F	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, CN, AK, F, SXT, CIP DO	> 32	1.5	OXA-48
165	*K. pneumoniae*	58Y	F	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, CIP, DO	> 32	16	OXA-48, NDM-1
166 F2	*K. pneumoniae*	58Y	F	Armpite	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, CIP, DO	> 32	> 32	NDM-1, VIM
106	*K. pneumoniae*	70Y	F	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP(I), ETP, AK, F, CIP	6	1	NDM-1
82	*K. pneumoniae*	(-)	F	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP(I), ETP, CIP	0.38	0.5	OXA-48
139	*K. pneumoniae*	(-)	(-)	Armpite	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	8	> 32	VIM
37	*K. pneumoniae*	(-)	F MS	HS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO IMP(I), ETP, F, SXT, CIP, DO	> 32	1.5	OXA-48
42	*K. pneumoniae*	(-)	FMS	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO IMP, ETP, AK, FF, F, CIP, DO	> 32	2	NDM-1
32	*K. pneumoniae*	(-)	FMS	HS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO IMP (I), ETP, AK (I), F, SXT, CIP, DO	> 32	2	NDM-1, VIM
16B	*K. pneumoniae*	70Y	M	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO IMP(I), ETP, AK(I)	1.5	> 32	Carba test (-)
G39'1'	*K. pneumoniae*	17Y	F	NS	MOW	H2	AX, AMC, TZP, FEP, KF, CRO IMP, ETP, AK, FF, F, CIP, DO	8	8	NDM-1
G48'1'	*K. pneumoniae*	6Y	M	NS	BSSR	H2	AX, AMC, TZP, FEP, KF, CRO IMP, ETP, FF, F, CIP, DO	8	8	NDM-1
G60	*K. pneumoniae*	28Y	F	TWS	FOW	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP	8	16	NDM-1
G78	*K. pneumoniae*	30Y	M	NS	BSSR	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, SXT, CIP, DO	8	16	NDM-1
G89	*K. pneumoniae*	33Y	M	WS	MOW	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, SXT, CIP, DO	8	24	NDM-1
G95	*K. pneumoniae*	29Y	M	TFC	NSR	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, SXT, CIP, DO	24	32	NDM-1
G97	*K. pneumoniae*	28Y	F	TWS	COW	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP, DO	24	32	NDM-1
G98	*K. pneumoniae*	24Y	M	FWS	BBSR	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP	6	16	OXA-48, NDM-1
G99	*K. pneumoniae*	74Y	F	WS	OPD	H2	AX, AMC, TZP, FEP, IMP, ETP, AK, CIP, DO	8	24	NDM-1
G100	*K. pneumoniae*	28Y	F	TWS	COW	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, FF, F, CIP, DO	16	> 32	OXA-48, NDM-1
G101	*K. pneumoniae*	28Y	F	TWS	COW	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP, DO	24	> 32	NDM-1
G102	*K. pneumoniae*	24Y	M	TWS	BSSR	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, FF, F, CIP, DO	24	> 32	OXA-48, NDM-1
BMC ICU1'1'	*K. pneumoniae*	3M	F	H/S	PW	H3	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, CN, FF, F, SXT, CIP, DO	24	> 32	OXA-48, NDM
BMC S8	*K. pneumoniae*	40Y	F	DF	FSW	H3	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, CN, F, SXT, CIP, DO	24	> 32	OXA-48, NDM-1
BMC S12	*K. pneumoniae*	68Y	M	DF	MSW	H3	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, CN(I), F, SXT, CIP, DO	4	8	OXA-48
BMC 16 GR'3'	*K. pneumoniae*	35Y	F	DF	FSW	H3	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, CN, FF, F, SXT, CIP, DO	> 32	> 32	OXA-48, NDM-1
BMC 48	*K. pneumoniae*	12D	F	BH/C	NICU	H3	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, SXT, CIP, DO	> 32	> 32	OXA-48, NDM-1

Slem 7894	*K. pneumoniae*	15D	F	Urine	PW	H3	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK (I), FF, F, SXT, CIP, DO	16	> 32	OXA-48
166 F1	*E. coli*	58Y	F	Armpit	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP(I), ETP, AK, CN, CIP, DO	4	6	NDM-1, OXA-48
138 F1	*E. coli*	75Y	F	NS	ICU	H1	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, AK, F, CIP, DO	2	6	OXA-48, NDM-1
BMC 28\27 GR 3	*C. freundii*	39Y	F	Wound	MSW	H3	AX, AMC, TPZ, FEP, KF, CRO, IMP (I), ETP, CN, SXT, DO	2	1.5	NDM-1
G32'1'	*C. freundii*	5Y	M	HWS	NNSR	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, FF, F, CIP	2	1	NDM-1
32	*C. freundii*	-	F MS	HS	ICU	H1	AX, AMC, TZP, FEP, KF, IMP (I), ETP	0.25	2	OXA-48
G56	*E. xiangfangensis*	45Y	M	FC	BSSR	H2	AX, AMC, TZP, FEP, KF, CRO, IMP, ETP, FF, SXT, CIP, DO	8	> 32	NDM-1

*BH/C: baby hand\cannula; BSSR: burn surgery shock room; COW: children orthopedic ward DF: diabetic foot; F: female; F MS; female medical staff; FC: Foley's catheter; FOW: female orthopedic ward; FSW: female surgical ward; FMS: female medical staff; H/S: hydrocephaly/sore; HS: hand swab; HWS: head wound; M: male; MOW: male orthopedic ward; MSW: male surgical ward; NICU: neonatal ICU; NS: nasal swab; NSSR: neurosurgical shock room; OPD: outpatient department; PW: peadiatric:; TFC: Tips of foley's catheter; TWS: thigh wound swab; WS: wound swab; **antibiotics**; AX: amoxicillin; AMC: amoxicillin/clavulanic acid; TPZ: ticarcillin/tazobactam; FEP: cefepime; KF: cephalothin; CRO: ceftriaxone; IMP: imipenem; ETP: ertapenem; AK: amikacin; CN: gentamycin; FF: fosofomycin; F: nitrofurantoin; SXT: trimethoprim/sulfamethoxazole; CIP: ciprofloxacin; DO: doxycycline.

Supplementary Table 2. Phenotypic and genotypic features of non-*Enterobacteriaceae* strains isolated from patients and medical staff of three Libyan hospitals.

Strain code	Strain ID	Age	Gender	Site	Ward	Hospital	Antibiotic susceptibility profile	E-test IMP	Carbapenemase gene and porin
16A	*A. baumannii*	70Y	M	NS	ICU	H1	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA(I), FF, F, SXT, CN, AK, CIP, DO	> 32	OXA-23
137	*A. baumannii*	75Y	F	HS	ICU	H1	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA(I), FF, F, SXT, CN, AK, CIP, DO	> 32	OXA-23
138 NF3	*A. baumannii*	75Y	F	NS	ICU	H1	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA(I), F, CN, AK, CIP, DO	> 32	NDM-1
G35	*A. baumannii*	20Y	M	NS	ICU	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, AK, CIP	> 32	OXA-23
G55	*A. baumannii*	35Y	M	TWS	ICU	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, CN, AK, RA(I), FF, SXT, CIP	> 32	NDM-1
G88	*A. baumannii*	17Y	F	FC	BSSR	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, CN, AK, RA(I), FF, SXT(I), CIP, DO	> 32	NDM-1
G90	*A. baumannii*	27Y	M	TETT	ICU	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, SXT, CIP	> 32	NDM-1
G91	*A. baumannii*	27Y	M	HWS	ICU	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, CN, AK, RA(I), FF, CIP	> 32	NDM-1
G92	*A. baumannii*	20Y	M	TFC	ICU	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, AK, CIP	> 32	OXA-23
G93	*A. baumannii*	28Y	M	BS	BSSR	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, AK, CN, SXT, CIP	> 32	NDM-1
G94	*A. baumannii*	29Y	M	TFC	NSR	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, SXT, AK, CN, CIP	> 32	OXA-23
G96	*A. baumannii*	30Y	M	TETT	ICU	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, AK, CN, SXT, CIP	> 32	OXA-23, NDM-1
BMC ICU 6'2'	*A. baumannii*	9M	F	H/S	PW	H3	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, CN, RA(I), FF, F, CIP	> 32	OXA-23
BMC S21	*A. baumannii*	58Y	M	Wound	MSW	H3	TIC, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, CN, SXT, DO	> 32	OXA-23, NDM-1
BMC 16 GR 3'3'	*A. baumannii*	35Y	F	DF	FSW	H3	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA(I), FF, F, AK(I), SXT, CIP, DO	> 32	NDM-1
BMC ICU 5	*A. baumannii*	14D	M	H/W	NICU	H3	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA(I), FF, CN, AK, CIP	> 32	(-)
G103	*P. aeruginosa*	32Y	M	AWS	BSSR	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, FF, AK, CIP, DO	> 32	OprD (substitution, deletion)
G104	*P. aeruginosa*	28Y	F	TWS	COW	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, AK, CN, SXT, CIP, DO	> 32	VIM-2
G31	*P. aeruginosa*	5Y	M	HWS	NSSR	H2	TIC, TIM, TPZ, ATM, CAZ, IMP, MEM, RA, AK, CN, SXT, CIP, DO	> 32	(-)
G42'2'	*P. aeruginosa*	12Y	F	NS	BSSR	H2	TIC, TIM, TPZ, CAZ, FEP, IMP, MEM, FF, SXT, AK, CN, CIP, DO	> 32	OprD (St'op codon)

G57	*P. aeruginosa*	6Y	M	AWS	BSSR	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, FF, AK, CN, SXT, CIP, DO	> 32	VIM-2
G58	*P. aeruginosa*	28Y	M	LWS	BSSR	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, FF, AK, SXT, CIP, DO	> 32	VIM-2
slem74650	*P. aeruginosa*	67Y	M	Urine	MSW	H3	TIC, TIM, TPZ, ATM(I), CAZ, FEP, IMP, MEM, RA, F, CN, AK, RA, SXT, CIP, DO	> 32	oprD (IS*Pa*26)
BMC 98	*P. aeruginosa*	4D	M	Armpit	NICU	H3	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, RA, F, FF, AK, CN, SXT, CIP, DO	> 32	VIM-2
G32	*P. oleovorans*	5Y	M	HWS	NSSR	H2	TIC, TIM, TPZ, FEP, IMP, MEM, RA, FF, SXT, CIP, DO	> 32	VIM-2
G48'2'	*P. mandocina*	6Y	M	NS	BSSR	H2	TIC, TIM, TPZ, CAZ, FEP, IMP, MEM, RA, FF, AK, DO	> 32	VIM-2
G37	*P. putida*	32Y	M	NS	ICU	H2	TIC, TIM, TPZ, ATM, IMP, MEM, RA, FF, SXT, CIP, DO	> 32	VIM-2
G40	*P. otitides*	18Y	F	NS	BSSR	H2	TIC, TIM, ATM, MEM, RA, FF, DO	> 32	VIM-2
156	*S.malthophila*	-	MS	NS	ICU	H1	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, CN, RA (I), FF, F	> 32	-

*AWS: abdominal wound swab; BS: blood sample; BSSR: burns surgery shock room; COW: children orthopaedic ward; D: day; DF: diabetic foot: F: female; FSW: female surgery ward; FC: Foley's catheter; HS: hand swab; H/S: hydrocephaly/sore: H/W: hydrocephaly/wound; HWS: head wound swab; LWS: leg wound swab; M: male; MSW: Male surgery ward; NSR: neurosurgery room; NICU: Neonatal ICU; NS: nasal swab; PW: Peadiatric ward; TFC: Tip of Foleys catheter; TETT: Tip of endotracheal tube; TWS: thigh wound swab; Y: year; antibiotics; TIC: Ticarcillin; TIM: Ticarcillin/clavulanic acid; TPZ: Piperacillin/tazobactam; ATM: Aztreonam; CAZ: Ceftazidime; FEP: Cefepime; IMP: Imipenem; MEM: Meropenem; RA: Rifampicin; FF: Fosofomycin; F: Nitrofurantoin; SXT: Trimethoprim/sulfamethoxazole; CN: Gentamycin; AK: Amikacin; CIP: Ciprofloxacin; DO: Doxycyclin.

Supplementary Table 3. Phenotypic and genotypic features of *Enterobacteriaceae* isolated from three Libyan hospital environmental settings.

Strain code	Strain ID	Site	Ward	Hospital	Antibiotic susceptibility profile	E-test IMP (µg/mL)	E-test ETP (µg/mL)	Carbapenemase -encoding gene
66	*K. pneumoniae*	Floor	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, AK, SXT, CIP, DO	3	> 32	OXA-48, NDM-1
97	*K. pneumoniae*	ICU Table	ICU	H1	AX, AMC, TPZ, FEP, KF(I), CRO, IMP, ETP, AK, F, CIP	3	4	OXA-48
145 F	*K. pneumoniae*	SM	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CN, AK, F, CIP, DO	4	6	OXA-48, NDM-1
160 A1	*K. pneumoniae*	Suction machine	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, AK, FF, F, SXT, CIP	2	> 32	NDM-1
124 A I	*K. pneumoniae*	WA	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, FF, F, CIP, DO	24	> 32	NDM-1
169 F	*K. pneumoniae*	BW	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP, DO	6	> 32	NDM-1
62	*K. pneumoniae*	PT	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, AK, F, CIP	1	6	NDM-1
126NF	*K. pneumoniae*	Floor	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, SXT, CIP, DO	24	32	NDM-1
127FA	*K. pneumoniae*	Floor	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, FF, F, AK, CIP, DO	4	4	NDM -1
48 F	*K. pneumoniae*	WT	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, AK, FF, F, SXT, CIP, DO	3	> 32	carba test (-)
159 NF3	*K. pneumoniae*	SM	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, FF, F, SXT, CIP, DO	6	12	carba test (-)
140	*K. pneumoniae*	PP	ICU	H1	AX, TPZ(I), CRO, IMP(I), ETP, CN, AK, F, SXT, CIP, DO	0.19	S	carba test (-)
G1	*K. pneumoniae*	WC	COW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	24	> 32	NDM-1
G2'2'	*K. pneumoniae*	DR	BSSR	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	> 32	> 32	OXA-48, NDM-1
G3	*K. pneumoniae*	WC	FOW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CIP	3	4	NDM-1
G4	*K. pneumoniae*	WC	MOW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, F, SXT, CIP, DO	4	> 32	NDM-1
G5'2'	*K. pneumoniae*	ER	CSR	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	4	6	NDM-1
G8'2'	*K. pneumoniae*	WC	MOW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, FF, F, AK, CN, CIP	2	24	NDM-1
G9	*K. pneumoniae*	WC	MSW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, CN, FF, F, CIP, DO	24	> 32	NDM-1
G11 '2'	*K. pneumoniae*	Bathroom	NSSR	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, AK, FF, F, SXT, CIP, DO	12	> 32	OXA-48, NDM-1

G14'2'	*K. pneumoniae*	WC	CSR	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP, DO	> 32	> 32	NDM-1
G17	*K. pneumoniae*	NR	FSW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP, DO	4	> 32	NDM-1
G18'2'	*K. pneumoniae*	WC	ICU	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, CIP, DO	4	12	NDM-1
G19	*K. pneumoniae*	WC	FSW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, FF, F, SXT, CIP, DO	24	> 32	NDM-1
G29'2'	*K. pneumoniae*	ER	COW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	> 32	> 32	NDM-1
G15 v	*K. pneumoniae*	WC	NSSR	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CIP	16	32	NDM-1
BMC 5	*K. pneumoniae*	Laryngscope	NICU	H3	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, CN(I), FF, F, SXT, CIP, DO	> 32	> 32	OXA-48, NDM-1
BMC 11	*K. pneumoniae*	IBI	NICU	H3	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	> 32	> 32	NDM-1
BMC 13	*K. pneumoniae*	HR	NICU	H3	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, AK, CN, SXT, CIP, DO	> 32	> 32	OXA-48, NDM-1
BMC 16	*K. pneumoniae*	BM	NICU	H3	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, AK, CN(I), SXT, CIP, DO	> 32	> 32	NDM-1, OXA-48
BMC B 36	*K. pneumoniae*	Sewage	NICU	H3	AX, AMC, TPZ, FEP; KF, CRO, IMP, ETP, F, AK, F, SXT, CIP, DO	-	> 32	OXA-48, VIM, NDM-1
BMC 35	*K. pneumoniae*	Sewage	NICU	H3	AX, AMC, TPZ, FEP; KF, CRO, IMP, ETP, FF, F, AK, CN(I), SXT, CIP, DO	> 32	> 32	NDM-1
BMC 47	*K. pneumoniae*	Sewage	UGF	H3	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, CN, FF, F, SXT, CIP, DO	> 32	> 32	OXA-48, NDM-1
G5'2'	*E. cloacae*	ER	CSR	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, CN, FF, F, SXT, CIP, DO	32	32	NDM-1
G6 rose	*E. cloacae*	DR	FOW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CN, FF, CIP	16	> 32	NDM-1
G12	*E. cloacae*	WC	MOW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, CN, F, SXT, CIP, DO	24	> 32	NDM-1
G14'1'	*E. cloacae*	WC	CSR	H2	AX, AMC, TPZ, FEP; KF, CRO, IMP, ETP, FF, F, AK, CN, SXT, CIP, DO	8	32	NDM-1
G18'1	*E. cloacae*	WC	ICU	H2	AX, AMC, TPZ, FEP; KF, CRO, IMP, ETP, FF, F, AK, CN, SXT, CIP, DO	8	> 32	NDM-1
G20	*E. cloacae*	DR	BPSW	H2	AX, AMC, TPZ, FEP; KF, CRO, IMP, ETP, FF, F, AK, CN, SXT, CIP, DO	6	32	NDM-1
G21	*E. cloacae*	UR	EOT	H2	AX, AMC, TPZ, FEP; KF, CRO, IMP, ETP, FF, F, AK, CN, SXT, CIP, DO	6	> 32	NDM-1
99	*E.cloacae*	Floor	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, F, SXT, CIP, DO	6	4	KPC-2
127 B	*E.cloacae*	Floor	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CIP, DO	2	2	OXA-48
124AT	*E. coli*	WA	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, CN, CIP	1	4	NDM-1, OXA-48
158NF2	*E. coli*	Suction	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP(I), ETP, CN, AK, CIP	1	3	NDM-1, OXA-48
G16'1'	*E. coli*	WC	FSW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CIP, DO	1.5	4	NDM-1
G54	*E. coli*	SA	plastic O. T	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, CIP, DO	4	4	OXA-48
127FA	*C. freundii*	Floor	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, SXT, CIP, DO	12	32	NDM-4
G22'2'	*R. ornithinolytica*	WC	FSW	H2	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, FF, AK(I), CIP	8	16	NDM-1
161 S	*S. marcescens*	SM	ICU	H1	AX, AMC, TPZ, FEP, KF, CRO, IMP, ETP, AK, F, CIP, DO	16	> 32	OXA-48

*BM: boiler machine; BPSW: burn and plastic surgery ward; BSSR: burn surgery shock room; BW: bathroom wall; COW: children orthopedic ward; CSR: Chest surgical room; DR: dressing room; EOT: emergency operation theatre; ER: examination room: HR: refrigerator handle; IBI: inside baby incubator; ICU: intensive care unit; FOW: female orthopedic ward; FSW: female surgical ward; MOW: male orthopedic ward; MSW: male surgical ward; NR: nursing room; NSSR: neurosurgical shock room; NICU: neonatal ICU; PP: patient pillow; PT: patient table; SA: scrub area; SM: suction machine; UGF: underground floor; UR: utility room; WT: water tap; WA: water aerator; WC: water closet; **antibiotics**; AX: amoxicillin; AMC: amoxicillin/clavulanic acid; TZP: ticarcillin/tazobactam; FEP: cefepime; KF: cephalothin; CRO: ceftriaxone; IMP: imipenem; ETP: ertapenem; AK: amikacin; CN: gentamycin; FF: Fosfomycin; F: nitrofurantoin; SXT: trimethoprim/sulfamethoxazole; CIP: ciprofloxacin; DO: doxycycline.

Supplementary Table 4. Phenotypic and genotypic characteristics of non-fermenting strains isolated from three Libyan hospital environmental settings.

Strain code	Strain ID	Site	Ward	Hospital	Antibiotic susceptibility profile	E-test IMP (µg/mL)	Carbapenemase-encoding genes and porins
159 NF4	*A. baumannii*	SE	ICU	H1	TIC, TCC, TPZ, ATM, CAZ, FEP, IMP, MEM, AK, CN, RA (I), FF, F, SXT, CIP, DO	> 32	OXA-23
107	*A. baumannii*	MV	ICU	H1	TIC, TCC, TPZ, ATM, CAZ, FEP, IMP, MEM, AK, CN, RA (I), FF, F, SXT, CIP, DO	> 32	OXA-23
143	*A. baumannii*	BM	ICU	H1	TIC, TCC, TZP, ATM, CAZ, FEP, IMP, MEM, AK, CN, RA (I), FF, F, CIP, DO	> 32	-
G11	*A. baumannii*	WC	NSSR	H2	TIC, TCC, TPZ, ATM, CAZ, FEP, IMP, MEM, AK, RA, FF, SXT, CIP	> 32	OXA-23
G80	*A. baumannii*	DT	ICU	H2	TIC, TCC, TPZ, ATM, CAZ, FEP, IMP, MEM, AK, CN, SXT, CIP, DO	> 32	OXA-23
G22'1'	*A.baumannii*	WC	FSW	H2	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, SXT, CN, AK, CIP, DO	> 32	OXA-23
BMC 45	*A. baumannii*	ST	NICU	H3	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, SXT, CN, AK, CIP, DO	> 32	OXA-23
BMC 46	*A. baumannii*	IBI	NICU	H3	TIC, TIM, TPZ, ATM, CAZ, FEP, IMP, MEM, FF, RA, CIP	> 32	NDM-1
160A2	*P. aeruginosa*	SE	ICU	H1	TIC, TCC, TPZ, ATM, CAZ, FEP, IMP, MEM, AK, RA, F, SXT, CIP, DO	> 32	VIM-2
158 NF2	*P. aeruginosa*	SE	ICU	H1	TIC, TCC, TPZ, ATM, CAZ, FEP, IMP, MEM, AK, CN, RA, F, SXT, CIP, DO	> 32	VIM-2
G15J	*P. aeruginosa*	WC	NSSR	H2	TIC, TCC, TPZ, ATM, CAZ, IMP, MEM, AK, SXT, DO	32	VIM-2
G16'2'	*P. aeruginosa*	WC	FSW	H2	TIC, TCC, ATM, CAZ, FEP, IMP, MEM, AK, CN, FF, SXT, CIP, DO	> 32	OprD (substitution, insertion)
G13	*P. aeruginosa*	WC	NSSR	H2	TIC, TCC, TPZ, CAZ, FEP, IMP, MEM, AK, CN, FF, SXT, CIP, DO	> 32	oprD (substitution)
G29'1'	*P. aeruginosa*	ER	COW	H2	TIC, TCC, TPZ, CAZ, FEP, IMP, MEM, AK, CN, FF, SXT, CIP, DO	> 32	OprD (stop codon 646 pb)
G30	*P. aeruginosa*	WC	Dr. R	H2	TCC, TPZ, ATM, FEP, IMP, MEM, RA, FF, SXT	> 32	VIM-2
G53	*P. putida*	SA	EOT	H2	TIC, TCC, ATM (I), CAZ, IMP, MEM, CN, RA (I), FF, SXT, DO	> 32	VIM-2
G23	*P. otitides*	WC	BPSW	H2	TIC, MEM, AK, FF, DO	-	-
G16'3'	*S. maltophila*	WC	FSW	H2	TIC, TCC, ATM, IMP, MEM, CN, RA	> 32	-
BMC A 36	*S. maltophila*	Sewage	NICU	H3	TIC, TIM, ATM, IMP, MEM, AK, FF	> 32	OXA-23
G7	*C. aquatica*	NR	FOW	H2	TIC, TCC, TPZ, ATM, CAZ, FEP, MEM, AK, FF, SXT, CIP	32	NDM-1

*BM: bed mattress; BPSW: burns and plastic surgery ward; COW: children orthopedic ward; DT: dressing trolley; Dr. R: doctors room; ER: examination room; EOT: emergency operation theater; FOW: female orthopedic ward; FSW: female surgical ward; IBI: inside baby incubator; ICU: intensive care unit; MV: mechanical ventilator; NICU: neonatal ICU; NR: nursing room; NSSR: neuro-surgical shock room; SA: scrubs area; SE: suction equipment; ST: suction table; WC: Water circle; **antibiotics;** TIC: ticarcillin; TCC: ticarcillin/ clavulanic acid; TPZ: piperacillin/ tazobactam; ATM: aztreonam; CAZ: ceftazidime; FEP: cefepime; IMP: imipenem; MEM: meropenem; AK: amikacin; CN: gentamycin; RA: rifampicin; FF: Fosfomycin; SXT: trimethoprim/ sulfamethoxazole; CIP: ciprofloxacin; DO: doxycycline.