Journal of Global Antimicrobial Resistance 18 (2019) 249–256

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Molecular drivers of emerging multidrug resistance in *Proteus mirabilis* clinical isolates from Algeria

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ARTICLE INFO

Article history:
Received 20 July 2018
Received in revised form 4 January 2019
Accepted 26 January 2019
Available online 21 February 2019

Keywords:
Proteus mirabilis
AmpC
Extended-spectrum β-lactamase
ESBL
Aminoglycoside-modifying enzyme
Algeria

ABSTRACT

Objectives: The aim of this study was to characterise the molecular drivers of multidrug resistance in *Proteus mirabilis* isolated from Algerian community and hospital patients.
Methods: A total of 166 *P. mirabilis* isolates were collected from two hospitals and eight private laboratories from four cities (Khemis Miliana, Aïn Defla, Oran and Chlef) located in northwestern Algeria. All isolates were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Antimicrobial susceptibility testing was performed by the disk diffusion and Etest methods. Genes encoding AmpC β-lactamases, extended-spectrum β-lactamases (ESBLs), quinolone resistance and aminoglycoside-modifying enzymes (AMEs) as well as plasmid replicon typing were characterised by PCR. Clonal relationships were also determined by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) typing and were compared with MALDI-TOF/MS proteomic typing.
Results: Of the 166 *P. mirabilis* isolates, 14 (8.4%) exhibited resistance to important antibiotics, including amoxicillin, amoxicillin/clavulanic acid, cefotaxime, gentamicin and ciprofloxacin, of which 4/14 (28.6%) had an ESBL genotype ($bla_{CTX\text{-}M\text{-}2}$) and 10 (71.4%) had an AmpC/ESBL genotype ($bla_{CMY\text{-}2}$/$bla_{TEM\text{-}1}$). AME genes were detected in all isolates, including *ant(2″)-I*, *aac(3)-I*, *aac(6′)-Ib*-cr and *aac(3)-IV*. The *qnrA* gene was identified in 13 isolates (7.8%). ERIC-PCR showed one predominant clone, with eight $bla_{CMY\text{-}2}$-producing isolates from UHC Oran belonging to profile A clustering together in the MALDI-TOF/MS dendrogram.
Conclusion: Here we report the first description of AME and plasmid-mediated quinolone resistance genes among ESBL- and/or AmpC β-lactamase-producing *P. mirabilis* isolates from community- and hospital-acquired infections in northwestern Algeria.

1. Introduction

Proteus mirabilis is an opportunistic pathogen belonging to the Enterobacteriaceae family. It is characterised by high motility on nutrient agar plates, known as swarming, which facilitates identification [1]. *P. mirabilis* can cause several types of infectious diseases in the community setting [1,2]. It is one of the most common causative agents of urinary tract infection [1,3]. Moreover, it is frequently associated with numerous nosocomial infections [3], including bloodstream infection [3,4], uncomplicated cystitis, pyelonephritis and prostatitis [5], neonatal meningoencephalitis [6], empyema [7] and diarrhoeal disease [8]. It is the second most frequently isolated Enterobacteriaceae, after *Escherichia coli*, in French hospitals [9].

P. mirabilis has the ability to display several mechanisms of antimicrobial resistance with the acquisition of extended-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase genes. Over the past few decades, numerous studies in different geographical areas of the world have described diverse genes

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https://doi.org/10.1016/j.jgar.2019.01.030
2213-7165/

encoding ESBLs and AmpC β-lactamases in *P. mirabilis* strains, such as CMY-16, CMY-12, VEB-6, TEM-67 and TEM-187 [10–14]. However, very few studies have investigated the epidemiology and molecular drivers of ESBL- and/or AmpC (CMY-2, CMY-4, TEM-1 and CTX-15)-producing *P. mirabilis* in Algeria [15,16]. The first AmpC β-lactamase (CMY-12) was identified in 2002 in a *P. mirabilis* hospital strain from an Algerian patient from Constantine city transferred to Hospital Saint-Antoine, France [11].

The initial mechanisms of fluoroquinolone resistance described in *P. mirabilis* were mutations of DNA gyrase and topoisomerase IV, encoded by the *gyrA*, *gyrB*, *parC* and *parE* genes [17]. Furthermore, there have been only a few reports describing the plasmid-mediated quinolone resistance (PMQR) genes *qnrB* and *qnrD* in *P. mirabilis* in Algeria [16,18]. Resistance of *P. mirabilis* to aminoglycosides has been reported in many parts of the world [19], however, it has never been studied in Algeria.

Acquisition of new genes plays a principal role in the evolution of antimicrobial resistance [13,14]. Recently, multiple antimicrobial resistance genes acquired by mobile DNA elements have emerged in *P. mirabilis* [12,13,20]. Mobile genetic elements are considered a key contributor in the transmission of novel antimicrobial resistance genes between clinical isolates as well as in the spread of multidrug-resistant (MDR) *P. mirabilis* strains, including the Tn*6450* multidrug resistance transposon [21], *Proteus* genomic island 1 (PGI1) [12] and Tn*1* transposon [13]. In addition, integrons in exogenous gene cassettes play an important role in the acquisition and lateral transfer of antimicrobial resistance in *P. mirabilis* [20].

Here we report the first description of the resistance mechanisms and molecular drivers in clinical *P. mirabilis* isolates from hospital- and community-acquired infections in northwestern Algeria.

2. Materials and methods

2.1. Identification of bacterial isolates

A total of 166 consecutive non-duplicate *P. mirabilis* were isolated during a 1-year period (January–December 2016) from several samples (urine, vaginal discharge, pus, etc.) at one University Hospital Center (UHC Oran), one Public Hospital Establishment (PHE Chlef) and eight private laboratories located in four different cities (Chlef, Aïn Defla, Khemis Miliana and Oran) in northwestern Algeria (Supplementary Table S1). All isolates were preliminarily identified using biochemical tests such as urea, indole and triple sugar iron (Institut Pasteur, Alger, Algeria) and AP20E (bioMérieux, Marcy-l'Étoile, France) and were confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) using a microflex LT MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) [22].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined on Mueller–Hinton agar (Becton Dickinson & Co., Pont-de-Claix, France) by the disk diffusion method using a panel of 17 antibiotics. In addition, minimum inhibitory concentrations (MICs) were determined by Etest for cefotaxime, ceftriaxone and amikacin according the criteria of the Antibiogram Committee of the French Society of Microbiology–European Committee on Antimicrobial Susceptibility Testing (CA-SFM-EUCAST) 2015 v.1.January.

ESBL production was assessed by the double-disk synergy test showing an enhancement of the zone of inhibition (champagne cork shape) between amoxicillin/clavulanic acid and cefotaxime, ceftriaxone, cefepime or aztreonam [23]. Moreover, all selected ESBL-producing isolates were confirmed using chromID® ESBL agar DRIG/MAC (bioMérieux).

2.3. Molecular analysis of antimicrobial resistance genes and sequencing

Total bacterial DNA for PCR amplification was obtained using an EZ1 DNA Extraction Kit (QIAGEN, Courtaboeuf, France) with an EZ1 Advanced XL biorobot (QIAGEN) according to the manufacturer's instructions.

PCR was conducted to characterise ESBL genes ($bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV}) [12], AmpC β-lactamase genes (AmpC1, AmpC/I and CMY-2) [10], AME genes [*aac(6′)-Ib*, *aac(3)-I*, *aac(3)-II/VI*, *aac(3)-III/IV*, *aac(6′)-I*, *aac(6′)-II*, *ant(2″)-I*, *ant(4′)-II* and *aph(3′)-VI*], 16S rRNA methylase genes (*rmtA*, *rmtB* and *armA*) [24,25], quinolone resistance genes (*gyrA*, *gyrB* and *parC*) [17] and PMQR genes (*qnrA*, *qnrB* and *qnrS*) [26]. Positive PCR products were submitted for sequenced using a BigDye™ Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequences were analysed using Codon Code Aligner software and were then identified using BlastN against the NCBI database (http://www.ncbi.nlm.nih.gov), and were confirmed with ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation).

2.4. Plasmid replicon typing

PCR-based replicon typing (PBRT) was performed to identify plasmid incompatibility (Inc) groups using simplex PCR as described by Carattoli et al. [27].

2.5. Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) typing and MALDI-TOF/MS proteomic typing

The epidemiological relationship of MDR *P. mirabilis* isolates was typed using ERIC-PCR with three different primers (ERIC1, ERIC2 [28] and ERIC2* [29]).

MALDI-TOF/MS proteomic typing of isolates was evaluated through analysis of spectrum and protein mass profiles [22], and the mean spectra projection (MSP) dendrogram was created using MALDI Biotyper 3.0 software (Bruker Daltonik). The clustering details of both dendrograms were evaluated on the basis of the arbitrary cut-off set up at a different distance levels: 540 and 1000 for the MSP dendrogram of 14 MDR *P. mirabilis* isolates; and 900 and 140 for the MSP dendrogram of all 166 *P. mirabilis* clinical isolates [22].

2.6. Statistical study

Epidemiological data were recorded in Microsoft Excel (Microsoft Corp., Redmond, WA). Statistical analysis was performed using OpenEpi v.3.01 software [US Centers for Disease Control and Prevention (CDC), Atlanta, GA] according to the CDC recommendations. A P-value of < 0.05 was considered statistically significant.

3. Results

3.1. Bacterial isolates and identification

During the 1-year study period, a total of 166 *P. mirabilis* were isolated from 166 patients, comprising 146 isolates from outpatients in PHE Chlef and eight private laboratories (Chlef, Aïn Defla and Khemis Miliana) and 20 isolates from inpatients in UHC Oran. Of the 166 isolates, 127 (76.5%) were collected from urine samples, 17 (10.2%) from pus and 22 (13.3%) from other specimens. The distribution of *P. mirabilis* according to patient sex, age and location of isolation is presented in Supplementary Table S1. All

isolates were identified using biochemical tests and AP20E and were confirmed by MALDI-TOF/MS, with a score values ranging from 2 to 2.5.

3.2. Antimicrobial susceptibility testing

Of the 166 *P. mirabilis* isolates, 111 (66.9%) were resistant to two or more antibiotics. Of these, only 16 isolates (9.6%) from UHC Oran and the community were found to produce β-lactamase according to the ESBL test (double-disk synergy test and chromID® ESBL agar DRIG/MAC).

Antimicrobial susceptibility testing showed that all of the *P. mirabilis* from UHC Oran were susceptible to aztreonam, imipenem and ertapenem, whilst the community isolates showed a low rate of susceptibility [1 (0.7%) to aztreonam, imipenem and ertapenem]. However, the UHC Oran isolates showed a slightly higher level of resistance to third-generation cephalosporins (cefotaxime and ceftriaxone) (9; 45.0%) compared with community isolates (7; 4.8%) ($P = 10^{-7}$). The situation was the same for fluoroquinolone (ciprofloxacin) resistance [9 (45.0%) in UHC Oran isolates compared with 8 (5.5%) in community isolates; $P = 3 \times 10^{-7}$]. The rate of aminoglycoside (gentamicin) resistance was also higher in UHC Oran isolates (10; 50.0%) compared with community isolates (25; 17.1%) ($P = 0.002$) (Table 1). In addition, community isolates had a higher level of resistance to the fourth-generation cephalosporin cefepime (22; 15.1%) compared with the second-generation cephalosporin cefoxitin (13; 8.9%) ($P > 0.05$). MICs ranged from 12 to 256 μg/mL for ceftriaxone and cefotaxime and from 4 to 8 μg/mL for amikacin. The results of antimicrobial susceptibility testing are shown in Table 1.

3.3. Molecular detection of extended-spectrum β-lactamase (ESBL) and ESBL/AmpC β-lactamase resistance genes

Fourteen ESBL- and ESBL/AmpC-producing isolates were selected for PCR analysis: 4/14 (28.6%) ESBL-producing isolates from the community carried only CTX-M-2 and 1/14 (7.1%) isolate co-produced CMY-2 in association with TEM-1 (ESBL/AmpC-producing). Moreover, 9/14 (64.3%) *P. mirabilis* isolates from UHC Oran produced CMY-2 with TEM-1, and 2 of them (14.3%) also carried the bla_{SHV-12} gene. Genotypic and phenotypic features of the 14 MDR *P. mirabilis* isolates are summarised in Table 2 and their geographical origins are shown in Fig. 1.

3.4. Analysis of fluoroquinolones and aminoglycoside resistance genes

Fluoroquinolones resistance was due to the presence of the *qnrA* gene in 13 isolates (92.9%) and was associated with the *aac (6′)-Ib*-cr gene in 1 community isolate and with *qnrS* in one UHC Oran isolate. *qnrB* was detected in only one community isolate (7.1%) (Table 2). No mutations in the *gyrA*, *gyrB* and *parC* genes were found in any of the isolates.

All isolates harboured at least one AME gene: 10 isolates (71.4%) harboured *aac(3)-IV*, including 8 isolates from UHC Oran and 2 isolates from the community, and 1 of the latter also harboured *aac (6′)-Ib*-cr; and one (7.1%) hospital isolate harboured *aac(3)-I*. Three community isolates (21.4%) were found to harbour *ant(2″)-I* and these isolates showed a high level of resistance to amikacin (Table 2).

3.5. Plasmid replicon typing

PBRT revealed that in community isolates plasmids carrying $bla_{CTX-M-2}$ belonged to the IncA/C, IncP and IncFII2 groups, whereas in UHC Oran isolates plasmid carrying bla_{CMY-2} and bla_{TEM-1} genes belonged to the IncA/C group only in two isolates (Table 2). No plasmid Inc groups were determined for eight hospital isolates harbouring bla_{CMY-2} and bla_{TEM-1} genes.

3.6. MALDI-TOF/MS proteomic typing and ERIC-PCR typing

MALDI-TOF/MS typing was used to type the epidemiological relationships of the 166 *P. mirabilis* isolates. According to the arbitrary at distance levels of 900 and 140, the MSP dendrogram showed a specific subcluster assembling the majority of ESBL/AmpC- and ESBL-producing isolates, as showed in Supplementary Fig. S1.

ERIC-PCR and MALDI-TOF/MS proteomic typing were used to type the clonal relationship of 14 MDR *P mirabilis* isolates. As shown in Fig. 2 and Table 2, three different genetic profiles were identified, comprising 8 (57.1%) MDR *P. mirabilis* isolates with ERIC profile A, 3 (21.4%) isolates with ERIC profile B and 3 (21.4%) isolates with ERIC profile C. According to the data generated by the similarity analysis, all isolates of clone type A were isolated from UHC Oran (inpatients) and were ESBL/AmpC-producing (CMY-2/TEM-1); most importantly, they were placed in the same cluster in the MALDI-TOF/MS dendrogram (cluster 2). Isolates of clone type B were isolated from the community (outpatients) and were

Table 1
Results of antimicrobial susceptibility testing of 166 *Proteus mirabilis* clinical isolates in Algeria.

Antimicrobial agent	No. (%) of resistant isolates		*P*-value
	Hospital isolates ($n = 20$)	Community isolates ($n = 146$)	
AMX amoxicillin	17 (85.0)	78 (53.4)	0.01
AMC amoxicillin/clavulanate	13 (65.0)	67 (45.9)	>0.05
TIC ticarcillin	10 (50.0)	35 (24.0)	0.02
FOX cefoxitin	9 (45.0)	13 (8.9)	0.00003
CTX cefotaxime	9 (45.0)	7 (4.8)	10^{-7}
CRO ceftriaxone	9 (45.0)	7 (4.8)	10^{-7}
FEP cefepime	6 (30.0)	22 (15.1)	>0.05
ATM aztreonam	0	1 (0.7)	–
IPM imipenem	0	1 (0.7)	–
ERT ertapenem	0	1 (0.7)	–
SXT sulfamthoxazole/trimethoprim	9 (45.0)	38 (26.0)	>0.05
GEN gentamicin	10 (50.0)	25 (17.1)	0.002
AMK amikacin	0	3 (2.1)	–
CIP ciprofloxacin	9 (45.0)	8 (5.5)	3×10^{-7}
FOS fosfomycin	10 (50.0)	37 (25.3)	0.04
RIF rifampin	13 (65.0)	55 (37.7)	0.03
COL colistin	20 (100)	146 (100)	–

AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; TIC, ticarcillin; FOX, cefoxitin; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; IPM, imipenem; ERT, ertapenem; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; FOS, fosfomycin; RIF, rifampicin; COL, colistin.

Table 2
Phenotypic and genotypic features of ESBL- and ESBL/AmpC β-lactamase-producing *Proteus mirabilis* clinical isolates ($n = 14$).

Code	Patient age (years)/sex	Specimen	Date of isolation	UHC service/ community	Resistance profile	Genotype				PBRT	ERIC clone
						AmpC	ESBL	PMQR	AME		
530	51/M	Urine	14/08/2016	Community[a]	AMX/AMC/CTX/CRO/FEP/ GEN/AMK/CIP		CTX-M-2, TEM-1,	*qnrA*, *aac (6′)-Ib-cr*	*aac (3)-IV*	FII2	C
591	52/F	Urine	09/07/2016	Community[b]	AMX/AMC/CTX/CRO/FEP/ GEN/AMK/CIP		CTX-M-2	*qnrB*	*ant (2″)-I*	A/C	B
1259	35/M	Pus	04/05/2016	Dermatology	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-I*	ND	C
1249	29/F	Urine	23/03/2016	Community[c]	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1, SHV-12	*qnrA*	*aac (3)-IV*	ND	C
994	28/F	Pus	04/04/2016	Dermatology	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-IV*	ND	A
924	74/M	Urine	23/03/2016	Surgery	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1	*qnrA*, *qnrS*	*aac (3)-IV*	A/C	A
1478	28/Child	Urine	25/05/2016	Internal medicine	AMX/AMC/CTX/CRO/FOX/ GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-IV*	ND	A
878	70/F	Abscess	16/05/2016	Reanimation	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-IV*	ND	A
971	26/M	Urine	21/03/2016	Surgery	AMX/AMC/CTX/CRO/FOX/ GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-IV*	ND	A
252	44/M	Urine	28/03/2016	Reanimation	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-IV*	A/C	A
844	59/M	Urine	22/03/2016	Reanimation	AMX/AMC/CTX/CRO/FOX/ FEP/GEN/CIP	CMY-2	TEM-1, SHV-12	*qnrA*	*aac (3)-IV*	ND	A
3126	62/M	Abscess	06/04/2016	Surgery	AMX/AMC/CTX/CRO/FOX/ GEN/CIP	CMY-2	TEM-1	*qnrA*	*aac (3)-IV*	ND	A
10807	4/F	Urine	22/09/2016	Community[b]	AMX/AMC/CTX/CRO/FEP/ GEN/AMK/CIP		CTX-M-2	*qnrA*	*ant (2″)-I*	A/C	B
1660	41/M	Urine	11/07/2016	Community[b]	AMX/AMC/CTX/CRO/FEP/ GEN/CIP		CTX-M-2	*qnrA*	*ant (2″)-I*	P	B

ESBL, extended-spectrum β-lactamase; UHC, University Hospital Center, Oran; AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; CTX, cefotaxime; CRO, ceftriaxone FEP, cefepime; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; FOX, cefoxitin; PMQR, plasmid-mediated quinolone resistance; AME, aminoglycoside-modifying enzyme; PBRT, plasmid-based replicon typing; ND, not determined; ERIC, enterobacterial repetitive intergenic consensus.
[a] Private laboratory in Khemis Miliana city.
[b] Private laboratory in Ain Defla City.
[c] Private laboratory in Chlef city.

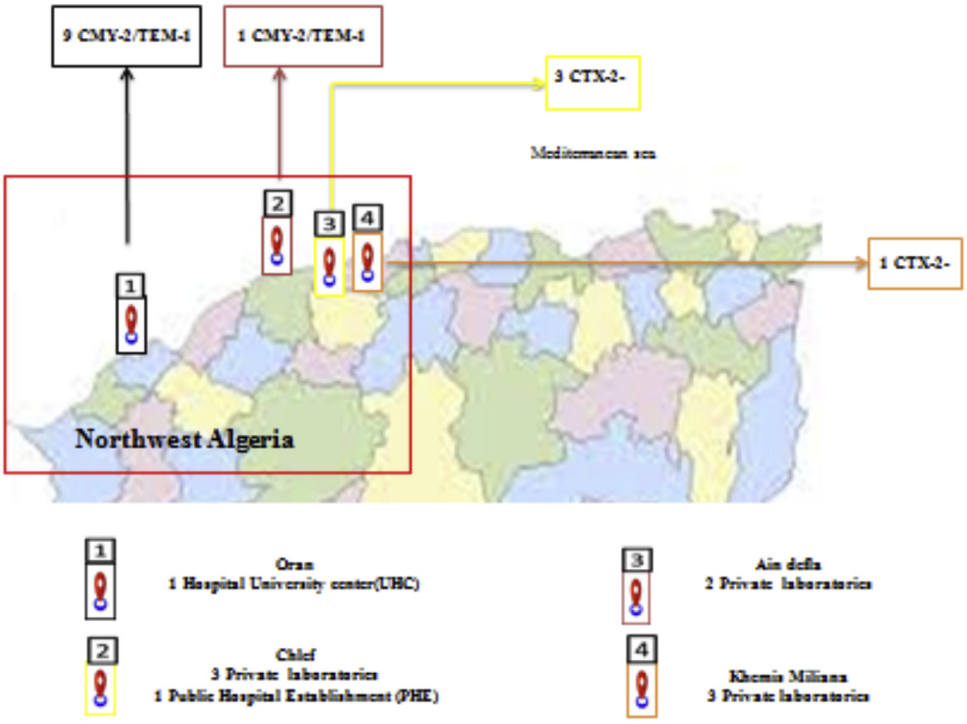

Fig. 1. Geographical origins of ESBL- and ESBL/AmpC β-lactamase-producing *Proteus mirabilis* isolates in northwestern Algeria. ESBL, extended-spectrum β-lactamase.

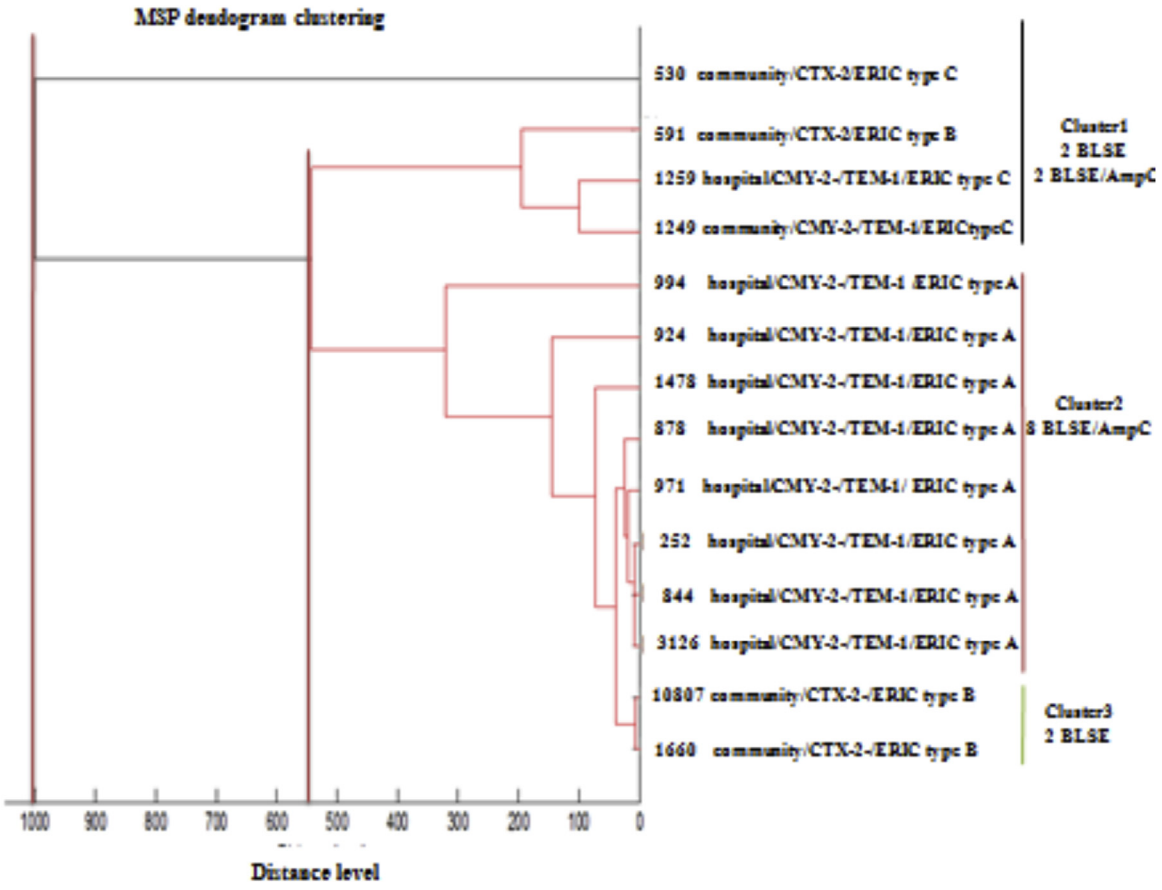

Fig. 2. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) proteomic typing according to the arbitrary cut-off set up at a distance levels of 540 and 1000 and its relationship with genotypic resistance and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) typing for 14 *Proteus mirabilis* isolates. MSP, mean spectra projection; ESBL, extended-spectrum β-lactamase.

ESBL-producers (CTX-M-2), whereas clone type C assembled one UHC Oran and two community isolates that were ESBL/AmpC-producers (CMY-2/TEM-1-) and ESBL-producers (CTX-M-2). Isolates of clone type C were grouped together in the MALDI-TOF/MS dendrogram (cluster 1) along with one isolate of clone type B, whereas the other two isolates of clones type B were separated into a different cluster 3.

4. Discussion

The emergence of MDR bacterial strains in hospital and community settings has been frequently reported as a major healthcare problem, especially β-lactamase-producing *P. mirabilis*, which could be considered the most important opportunistic pathogen responsible for nosocomial and community infections [2,3].

ESBL- or AmpC/ESBL-producing *P. mirabilis* strains have been related to several health problems, including chronic infections, antibiotic treatment failure and mortality [4], thus increasing the demand for epidemiological monitoring. In Algeria, very few studies have been conducted to characterise the molecular drivers in MDR *P. mirabilis* isolates of human origin.

The results of this study demonstrated a low rate of ESBL-producing *P. mirabilis* (4.8%) in the Algerian community compared with that reported in a Tunisian community from 2010 to 2013 (9%) [30]. This is not the case for the European community, including Spain, Germany and Belgium, which reported a low rate of ESBLs (1.2%) between August 2013 and January 2014 [31]. However, the prevalence of ESBL/AmpC-producing *P. mirabilis* in an Algerian UHC (45%) was very high compared with the rate in a Ugandan UHC (11.4%) between July 2016 and September 2016 [32] and in a French UHC (6.9%) from 1996 to 1998 [9].

This is likely related to empirical antibiotic use by Algerian healthcare clinicians in Oran UHC and outpatient care services that resulted in selective pressure followed by spread to extraclinical and intraclinical environments. Accordingly, 9 (64.3%) of 14 isolates from urine samples showed an ESBL/AmpC phenotype, in agreement with previous studies showing an association between ESBL/AmpC-producing *P. mirabilis* and urinary tract infections [2,3,30,31].

The epidemiological findings showed that resistance to β-lactams was mainly due to the presence of the bla_{CMY-2} AmpC β-lactamase gene associated with the presence of the bla_{TEM-1} ESBL gene in hospitals and the community, which has never been detected to date among *P. mirabilis* isolates in Algeria. There is only one description of TEM-1 associated with CMY-4 in *P. mirabilis* from Béjaïa Hospital in eastern Algeria [16], whereas CMY-2 has already been detected with CTX-15 in one clinical isolate from Algiers Hospital in northern Algeria [15]. We also exclusively detected the bla_{SHV-12} gene associated with bla_{CMY-2} and bla_{TEM-1} in two *P. mirabilis* isolates from UHC Oran and the community, respectively. Nevertheless, it has already been described in several clinical isolates of *Klebsiella pneumoniae* and *E. coli* collected from Algerian hospitals [15,16]. The current study reports the first

identification of CTX-M-2, which is the predominant ESBL genotype, found among *P. mirabilis* isolates from the Algerian community, although it was previously detected in one *E. coli* isolate in Algeria [33] and in *P. mirabilis* isolates in Japan [34].

To the best of our knowledge, we report for the first time the presence of *qnrS* and *qnrA* genes in *P. mirabilis* isolates in Algeria, which were previously detected in Algerian *Enterobacter cloacae* isolates [35], in Algerian *E. coli* [18] and in French *P. mirabilis* [12]. *qnrB* was previously described in Algerian *P. mirabilis* isolates from the community [16,18] and in *E. cloacae* from hospitals [35]. To the best of our knowledge, this is the first detection of the *aac(6′)-Ib-cr*, *ant(2″)-I* and *aac(3)-I* genes conferring aminoglycoside resistance in *P. mirabilis* isolates from the Algerian community, although they have been recently reported in *E. cloacae* isolates from UHC of Annaba City in the east of Algeria [35] as well as in *K. pneumoniae* from the community of Béjaïa city in east Algeria [16].

This study highlights the first report of the occurrence *aac(3)-IV* genotype in *P. mirabilis* from Algeria and Africa in ten clinical isolates, which was also recently found in *E. coli* and *K. pneumoniae* isolated from wild fish in the Mediterranean Sea in Béjaïa city [36].

The aminoglycoside 3-*N*-acetyltransferase gene *aac(3)-IV* confers resistance to most of the aminoglycoside family, mainly apramycin and gentamicin, called the apramycin resistance gene [37], which is rarely detected in clinical isolates. The plasmid-encoded *aac(3)-IV* gene is found mostly in bacteria of animal origin [37], suggesting that this resistance gene could be transferred to humans via direct contact with animals carrying MDR *P. mirabilis* isolates or could be transported through the environment (e.g., vegetables, fruit, meat or water) [22,33,36]. This suggests that the environment can be considered as a principal reservoir of *aac(3)-IV* to date in Algeria and an important route for spread to humans [36].

It is important to note that the occurrence of *aac(3)-IV* in new MDR *P. mirabilis* clones containing multiple resistance genes such as ESBL/AmpC or ESBL genotype [*aac(3)-IV* + $bla_{\text{CMY-2}}$/$bla_{\text{TEM-1}}$ or $bla_{\text{CTX-M-2}}$) associated with a PQMR genotype involves new emerging resistance mechanisms disseminated in clinical isolates that were detected exclusively in this study. According to the literature, the linkage of an AME-encoding gene [*ant(2″)*-I] with an ESBL gene ($bla_{\text{CTX-M-2}}$) and a PQMR gene (*qnrA*) in three *P. mirabilis* isolates isolated from two different cities of the Algerian community could be associated with a worrisome clone that has not been detected until now, which poses a great risk to human health.

It is recognised that *P. mirabilis* is intrinsically resistant to colistin (polymyxin E). Its association with a large variety of acquired resistance mechanisms, including ESBL/AmpC genes and AME genes as well as quinolone resistance genes (*qnrA*/*qnrB*/*qnrS*), displays a complex clinical concern related to a decreased choice of effective antibiotics; indeed, the carbapenem group is the only antibiotic agent that could be used to treat infectious diseases, leading to the occurrence of carbapenem resistance genes in *P. mirabilis* and other bacterial species [12,38], as described in a recent epidemiological report from Algeria, where $bla_{\text{OXA-48}}$ was one of the most widespread carbapenemase-encoding gene detected among Enterobacteriaceae [38]. For this reason, the requirement for new antimicrobial measures should be applied under the supervision of Algerian physicians to prevent further dissemination of antimicrobial resistance genes, particularly in *P. mirabilis*.

To our knowledge, this is the first description of IncP- and IncFII2-type plasmids associated with the $bla_{\text{CTX-M-2}}$ gene in *P. mirabilis* community isolates. This gene has only been reported in *P. mirabilis* isolates in Japan localised on a transferable IncT plasmid [39]. In the current study, an IncA/C plasmid associated with $bla_{\text{CTX-M-2}}$ was also detected in two *P. mirabilis* community isolates for the first time in Algeria, which had already been observed in Enterobacteriaceae isolates in Brazil [40].

In the last decades, several investigations have described the IncA/C-type plasmid, which is the main mechanism involved in the spread of the $bla_{\text{CMY-2}}$ cephalosporinase gene [20,39]. It has been frequently identified in many regions, including the USA, China and France [20,39]. Indeed, we identified the IncA/C plasmid linked to the $bla_{\text{CMY-2}}$ gene in two nosocomial *P. mirabilis* isolates, which has been described in a previous study from Algeria [15]. However, we could not determine the location of the $bla_{\text{CMY-2-}}$ gene in eight isolates. It may be harboured by an integrative conjugative element (ICE) such as SXT/R391 [39] or a specific transposon-like element such as IS*Ecp1* responsible for the spread of $bla_{\text{CMY-2}}$ among members of the Enterobacteriaceae family [20] or via another unknown mechanism requiring further study.

The MSP dendrogram of *P. mirabilis* isolates in Supplementary Fig. S1 revealed a specific subcluster including only ESBL- or ESBL/AmpC-producing isolates, confirming the results obtained by Nour el Houda Khennouchi et al. and Djeffal et al. [22,35] who showed that the MSP dendrogram is a useful technique for classification of bacterial isolates based on their resistance phenotypes.

The MSP dendrogram of 14 MDR *P. mirabilis* isolates (Fig. 2) showed that the isolates in the current study were separated in three clusters according to their specific resistance genotype (ESBL or ESBL/AmpC), and their clinical origins (community/UHC), which can be important for rapidly identifying sources of isolates and their molecular drivers [22,33].

ERIC-PCR revealed that MDR *P. mirabilis* isolates were separated in three different clonal types. This genotyping method is currently considered as a powerful tool for epidemiological analysis [28,29] based on the position of the ERIC sequence in enterobacterial genomes. Indeed, eight *P. mirabilis* isolates belonging to the same clone type A isolated from UHC Oran and having the same resistance genotype [$bla_{\text{CMY-2}}$/$bla_{\text{TEM-1}}$/*qnrA*/*AAC(3)-IV*] were separated in a distinct cluster (cluster 2) in the MALDI-TOF/MS dendrogram [22,33]. This specific cluster suggests the circulation of *P. mirabilis* isolates belonging to clone type A between different services of UHC Oran (reanimation/surgery) (Table 2).

In clonal type B, the presence of the same incompatibility group IncA/C plasmid in two *P. mirabilis* isolates derived from two cities and two different laboratories was observed. This may be due to the circulation of the current transmissible plasmid in the community population between two different cities or via another unknown mechanism.

At the same time, clone type C consisted of two community isolates and one UHC isolate, located in the same cluster in MALDI-TOF/MS analysis (cluster 1) and with different resistance genotypes. This reflects a higher rate of intrinsic evolution given the diversity of genotype in *P. mirabilis* isolates from northwestern Algeria.

In conclusion, we report for the first time the emergence of AME-encoding genes along with ESBL/AmpC-encoding and PQMR genes in *P. mirabilis* clinical isolates from UHC Oran and from community settings in four cities located in northwestern Algeria. It is of note that full resistance of *P. mirabilis* to β-lactams, aminoglycosides and quinolones, coming both from acquisition of multiresistance plasmids or chromosomally-encoded genes, combined with species-inherent resistance to polymyxins represents extremely resistant strains that can be rapidly disseminated in clinical and external environments.

Acknowledgments

The authors are very grateful to the chiefs of service Amina Faraji and Moustapha Belaid at the microbiology laboratory of Hospital Merouane Abed de Chettia (Chlef, Algeria) for their

technical help. The authors would like to thank Prof. Jean-Marc Rolain (IHU Méditerranée Infection, Marseille, France) for excellent technical assistance and for the constructive comments and recommendations.

Funding

This work was partly supported by the French Government under the 'Investments for the Future' program managed by the National Agency for Research (ANR), Méditerranée Infection 10-IAHU-03.

Competing interests

None declared.

Ethical approval

All laboratories gave official permission to collect *Proteus mirabilis* isolates.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2019.01.030.

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