Molecular epidemiology and antimicrobial resistance patterns of Clostridioides difficile isolates in Algerian hospitals

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Abstract

Introduction: Clostridioides difficile is a major pathogen responsible for hospital-associated diarrhoea. This study investigated the molecular epidemiology and antibiotic resistance of C. difficile isolates in five Algerian hospitals.

Methodology: Between 2016 and 2019, faecal specimens were collected from in-patients and were cultured for C. difficile. Isolates were characterised by toxin genes detection, Polymerase Chain Reaction (PCR)-ribotyping, Multilocus Sequence Typing (MLST), antimicrobial susceptibility testing against a panel of antibiotics, and screened for antimicrobial resistance genes.

Results: Out of 300 patient stools tested, 18 (6%) were positive for C. difficile by culture, and were found to belong to 11 different ribotypes (RT) and 12 sequence types (ST): RT 085/ST39, FR 248/ST259, FR 111/ST48, RT 017/ST37, RT 014/ST2, RT 014/ST14, FR 247/new ST, RT 005/ST16, RT 029/ST16, RT 039/ST26, RT 056/ST34 and RT 446/ST58. MLST analysis assigned the isolates to two clades, 1 and 4. Clade 4 was more homogeneous, as it mainly included non-toxigenic isolates. Three toxin gene profiles were detected, two toxigenic, A+B+CDT- (33.3%) and A-B+CDT- (11%); and one non-toxigenic, A-B-CDT- (55.5%). All C. difficile isolates were susceptible to metronidazole, vancomycin and moxifloxacin.

Conclusions: Overall prevalence of C. difficile in our healthcare settings was 6%. Antibiotic resistance rates ranged from 72.2% (clindamycin) to 16.6% (tetracycline). This study highlighted a relatively high genetic diversity in term of ribotypes, sequence types, toxin and antibiotic resistance patterns, in the C. difficile isolates. Further larger studies are needed to assess the true extent of C. difficile infections in Algeria.

Key words: Clostridioides difficile; Algeria; ribotyping; MLST; antibiotic resistance; toxins.


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resistant strains, such as the epidemic NAP1/BI/027 strain [5]. Compared to Europe and North America, CDI is a largely neglected disease in the developing countries and epidemiological data on C. difficile are scarce or lacking. In Africa, the highest prevalence of C. difficile was reported in Kenya (93.3%) [6] and the lowest in the Ivory-Coast (2%) [7], whereas in the Middle-East, the highest prevalence was recorded in Lebanon (82.9%) [8], and the lowest in Kuwait (0.5%) [9]. In Algeria only one study was conducted reporting a prevalence of 6.9% [10]. The wide variability in the above prevalence estimates is presumably due to a combination of factors such as study designs, type of population studied and C. difficile identification methods.

The aim of this study was to investigate the molecular epidemiology and antibiotic resistance of C. difficile in five hospitals located in three different provinces of Algeria.

**Methodology**

**Study design and sample collection**

Unformed stool samples were collected from patients admitted to five hospitals in three different provinces, Batna, Ain Defla and Chlef; located in the East, Centre and West of Algeria, respectively, between January 2016 and January 2019. All patients included in the study developed diarrhoea, defined as 3 or more loose or liquid stools per day, or more frequently than is normal for the individual (as defined by the World Health Organization, http://www.who.int/topics/diarrhoea) [11], after three days of admission with or without previous antibiotic treatment. Infants under the age of 2 years old are excluded from this study due to the high asymptomatic carriage of C. difficile in this group [12].

**C. difficile culture and identification**

Following alcohol-treatment of the stool samples to eliminate vegetative cells [13], the remaining spores were cultured on a selective medium (Clostridium difficile chromogenic agar; ChromID CDIF, Biomérieux, Marcy l’Etoile, France). The plates were incubated at 37 °C for 48 h under anaerobic conditions (10% H2, 2.5% CO2, 85% N2) using AnaeroGen 2.5L (Thermo scientific, Tokyo, Japan). Black colonies or suspicious non-black colonies (based on the morphological aspect) were identified using Matrix Assisted Laser Desorption Ionisation-Time Of Flight mass spectrometry (MALDI-TOF MS) (Microflex LT BRUKER, Madison, USA).

**Molecular identification and toxin genes detection**

Genomic DNA was extracted using InstaGene Matrix Kit (Bio-Rad, Hercules, USA), following the manufacturer's instructions. C. difficile isolates were stored at -80 °C using Microbank mixed microbial storage vials (Pro-Lab diagnostics, Ontario, Canada).

For molecular characterisation of C. difficile isolates and toxin genes, a multiplex PCR assay was carried out according to the protocol of Barbut et al., 2019 [14], using seven pairs of primers targeting the following genes: tpi, (triose phosphate isomerase), tcdA (toxin A), tcdB (toxin B) cdtA and cdtB (binary toxin subunits), the PaLoc and tcdC (negative regulator for toxin expression) [15]. C. difficile PCR-ribotype (RT) 027, was used as positive control. The amplicons were analysed using a high-resolution capillary electrophoresis detention system (HITACHI ABI 3500 Genetic Analyzer, Applied Biosystems, Massachusetts, USA). The results were visualised using GeneMapper Software version 5.0 (Applied Biosystems, Massachusetts, USA).

**Polymerase Chain Reaction (PCR)-ribotyping**

PCR-ribotyping was performed according to the protocol recommended by The European Centre for Disease Prevention and Control (ECDC), using primers designed by Bidet et al [16]. For the amplification of the 16S-23S rRNA intergenic region, a capillary electrophoresis was performed using a Genetic Analyser (HITACHI ABI 3500, Applied Biosystems, Massachusetts, USA) and electrophoreograms were visualised using GeneMapper Software version 5.0 (Applied Biosystems, Massachusetts, USA). PCR-ribotype (RT) was determined using WEBRIBO database version 2.2 available at: https://webribo.ages.at/. When PCR-ribotyping profiles are unknown, the prefix “FR” was used (French reference laboratory internal nomenclature).

**Multiilocus sequence typing (MLST)**

MLST was performed as described by Griffiths et al [17], using PCR primers targeting seven housekeeping genes (adk, atpA, ddx, glyA, recA, sodA and tpi). The sequence type (ST) and clade were determined by comparing the sequences of strains with the MLST database (http://pubmlst.org/cdifficile).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility to clindamycin (CLD), erythromycin (ERY), moxifloxacin (MXF) and tetracycline (TET) was assessed using the disk diffusion method (I2A, France). For metronidazole
(MTZ) and vancomycin (VAN), the minimal inhibitory concentration (MIC) was determined using an E-test (Biomerieux, Marcy l’Etoile, France). Brucella blood agar plates supplemented with 0.5 mg/L hemin, 1mg/L Vitamin K1 and 5% sheep blood (Becton Dickinson GmbH, Heidelberg, Germany) were inoculated with 1.5 MacFarland bacterial suspension. Plates were then incubated for 48 h at 37 °C in an anaerobic atmosphere using AnaeroGen 2.5L (Thermo scientific, Tokyo, Japan). The diameter of inhibition zones was interpreted according to the CA-SFM 2019 recommendations (Antibiotic susceptibility committee of the French society for microbiology) [18]. Breakpoints were set as follows: TET (30 μg) < 19 mm, MXF (5 μg) < 21 mm, CLD (2 UI) < 15 mm, ERY (15 UI) < 22 mm [18]. For MTZ and VAN, MIC breakpoint 2 mg/L was applied as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19]. Multidrug-resistance was considered when the strain showed resistance to 3 or more antimicrobial classes.

Detection of antibiotic-resistance genetic determinants

The presence of the resistance genetic determinants for the macrolide-lincosamide-streptogramin B (MLSb) family of antibiotics (ermB), tetracycline (tetM, tetO, tetB[P]), tetO/32/0, tet40), tetA[P]) [20] and fluoroquinolones (gyrA) mutations in the quinolone resistance-determining region (QRDR) was investigated as previously described [20,21].

**Table 1.** Molecular characterisation of *C. difficile* isolates in the study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Province</th>
<th>Hospital</th>
<th>Ward</th>
<th>Year</th>
<th>Gender</th>
<th>Age</th>
<th>Genotyping</th>
<th>Ribotype</th>
<th>Sequence type (Clade)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 038</td>
<td>Batna</td>
<td>UH</td>
<td>MIW</td>
<td>2016</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 053</td>
<td>Batna</td>
<td>UH</td>
<td>MIM</td>
<td>2016</td>
<td>M</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 053 26 (1)</td>
</tr>
<tr>
<td>CD 093</td>
<td>Batna</td>
<td>UH</td>
<td>REA</td>
<td>2016</td>
<td>M</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 137</td>
<td>Batna</td>
<td>UH</td>
<td>PED</td>
<td>2017</td>
<td>F</td>
<td>Ch</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 144</td>
<td>Batna</td>
<td>UH</td>
<td>PED</td>
<td>2017</td>
<td>M</td>
<td>Ch</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 147</td>
<td>Batna</td>
<td>UH</td>
<td>MIW</td>
<td>2017</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
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<td>Batna</td>
<td>UH</td>
<td>MIM</td>
<td>2017</td>
<td>M</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 181</td>
<td>Batna</td>
<td>UH</td>
<td>PED</td>
<td>2017</td>
<td>F</td>
<td>Ch</td>
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<td>RT 085 39 (4)</td>
</tr>
<tr>
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<td>MIW</td>
<td>2017</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
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</tr>
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<td>UH</td>
<td>MIW</td>
<td>2017</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
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<tr>
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<td>MIW</td>
<td>2017</td>
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<td>Ad</td>
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<td>+</td>
<td>RT 085 39 (4)</td>
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<tr>
<td>CD 213</td>
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<td>UH</td>
<td>MIM</td>
<td>2017</td>
<td>M</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 250</td>
<td>Chlef</td>
<td>Chettia</td>
<td>MIW</td>
<td>2018</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 213</td>
<td>Chlef</td>
<td>Chettia</td>
<td>PED</td>
<td>2018</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 250</td>
<td>Batna</td>
<td>UH</td>
<td>MIW</td>
<td>2018</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 204</td>
<td>Ain Defla</td>
<td>S/Bobida</td>
<td>PED</td>
<td>2018</td>
<td>M</td>
<td>Ch</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 207</td>
<td>Ain Defla</td>
<td>S/Bobida</td>
<td>PED</td>
<td>2018</td>
<td>M</td>
<td>Ch</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 025</td>
<td>Chlef</td>
<td>Chorfa</td>
<td>PED</td>
<td>2018</td>
<td>F</td>
<td>Ch</td>
<td>-</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
<tr>
<td>CD 50</td>
<td>Batna</td>
<td>UH</td>
<td>MIW</td>
<td>2018</td>
<td>F</td>
<td>Ad</td>
<td>+</td>
<td>+</td>
<td>RT 085 39 (4)</td>
</tr>
</tbody>
</table>

**Statistical analyses**

The data were coded using Excel 2013 (Microsoft, Redmond, WA, USA) and analyzed by R software (R Development Core Team, 2016). The distribution of prevalence of *C. difficile* between provinces, ages and sexes of patients was tested by Chi-Square test or Fisher's exact test. A level of *p value* < 0.05 was considered as statistically significant.

**Results**

**Prevalence rates of *C. difficile***

This multi-center, prospective study included a total of 300 patients admitted to five hospitals in three provinces of Algeria over a period of three years. The majority of patients were from the province of Batna (n = 258, 86%, 1 university hospital), followed by Chlef (n = 31, 10.3%, 3 hospitals) and Ain Defla (n = 11, 3.7%, 1 hospital). There were more females (n = 168, 56%) than males (n = 132, 44%); the majority of patients (n = 237, 79%) were adults (≥ 19 years old), and 21% (n = 63) were ≤ 18 years old.

Of the 300 patients, a total of 18 *C. difficile* isolates were cultured, giving an overall prevalence of 6% (CI at 95%: 3.3%-8.7%). The highest prevalence was recorded in the province of Ain Defla (18.2%, 2/11), followed by Chlef (9.7%, 3/31) and Batna (5%, 13/258). The prevalence was higher (11.1%, 7/63) in patients who were ≤ 18 years old than those aged ≥ 19 years old (4.6%, 11/237). The prevalence in females (6.5%, 11/168) was slightly higher than in men (5.3%, 7 /132). However, the differences in *C. difficile*...
prevalence between the three provinces, genders and age groups were not statistically significant (p value > 0.05).

Detection of tcdA, tcdB, cdtA/B and tcdC genes

A PCR multiplex assay for the detection of tcdA, tcdB and cdtA/B genes, revealed the presence of three toxin genes profiles: six C. difficile isolates (33%) with intact tcdA and tcdB, deleted cdtA and the cdtB as a pseudogene (A+B+CDT-); two C. difficile isolates (11%) revealed a deletion in tcdA and intact tcdB, deleted cdtA and cdtB (A-B+CDT-); the remaining ten C. difficile isolates (55.5%) did not carry any of the toxin genes (A-B-CDT-) (Table 1). The same analysis revealed that the tcdC gene was present (without internal deletion) in all the toxigenic isolates and absent in all the non-toxigenic isolates.

PCR ribotyping and multilocus sequence typing

The 18 C. difficile isolates were assigned to 11 different ribotypes and 12 sequence types: RT 085/ST39 (n = 3), FR 248/ST259 (n = 3), FR 111/ST48 (n = 2), RT 017/ST37 (n = 2), RT 014/ST2 (n = 1), RT 014/ST14 (n = 1), FR 247/new ST (n = 1), RT 005/ST6 (n = 1), RT 029/ST16 (n = 1), RT 039/ST26 (n = 1) and RT 056/ST34 (n = 1), FR 248/ST259 (n = 3), FR 111/ST48, RT 005/ST6, RT 14/ST2, RT 14/ST14, RT 029/ST16, RT 039/ST26, RT 056/ST34, FR 111/ST48, RT 446/ST58 and FR 247/New ST; whereas clade 4 included RT 85/ST39, RT 17/ST37 and FR 248/ST259. In addition, clade 4, include mainly non-toxigenic isolates, with the exception of 2 isolates belonging to RT 17/ST37, which produce toxin B only.

Detection of antimicrobial susceptibility and antibiotic-resistance genes

Antibiotic-susceptibility data of the 18 C. difficile isolates are presented in Table 2. All isolates were susceptible to MTZ and VAN, the first line of antibiotics used for the treatment of CDI, and to MXF, a fourth-generation fluoroquinolone. Sequence analysis showed no substitution in the QRDR of gyrA of all our isolates.

Five isolates (27.7%, 5/18) belonging to RT 005 (n = 1), RT 039 (n = 1), RT 085 (n = 1) and FR 111 (n = 2) were resistant to the MLSB family antimicrobials CLD and ERY, conferred by the presence of the ermB gene.

A similar resistance rate against the macrolide antibiotics CLD was found (27.7%, 5/18) in the isolates

<table>
<thead>
<tr>
<th>RT Stains</th>
<th>ATB ( mm)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TET</td>
<td>CLD</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>&lt; 15</td>
</tr>
<tr>
<td>RT 085</td>
<td>038</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>93</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>137</td>
<td>S</td>
</tr>
<tr>
<td>FR 248</td>
<td>C13</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>D04</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>D07</td>
<td>S</td>
</tr>
<tr>
<td>RT 014</td>
<td>155</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>181</td>
<td>R+</td>
</tr>
<tr>
<td>RT 017</td>
<td>F2</td>
<td>R+</td>
</tr>
<tr>
<td></td>
<td>S0</td>
<td>R+</td>
</tr>
<tr>
<td>FR 111</td>
<td>144</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>S</td>
</tr>
<tr>
<td>RT 005</td>
<td>213</td>
<td>S</td>
</tr>
<tr>
<td>RT 029</td>
<td>C05</td>
<td>S</td>
</tr>
<tr>
<td>RT 039</td>
<td>053</td>
<td>S+</td>
</tr>
<tr>
<td>RT 056</td>
<td>190</td>
<td>S</td>
</tr>
<tr>
<td>RT 446</td>
<td>210</td>
<td>S</td>
</tr>
<tr>
<td>FR 247</td>
<td>202</td>
<td>S</td>
</tr>
</tbody>
</table>

RT: ribotype; ATB: antibiotic; R: resistant; S: susceptible; TET: tetracycline; MXF: moxifloxacin; ERY: erythromycin; CLD: clindamycin; MTZ: metronidazole; VAN: vancomycin; MIC: minimal inhibition; concentration; +: presence of the gene; -: absence of the gene.
of the ribotypes RT 29 (n = 1), RT 085 (n = 1) and FR 248 (n = 3); one of which (RT 29) was not found to carry the *ermB* gene.

Two RT 085 (n = 1) and FR 247 (n = 1) isolates were resistant to ERY only. The latter isolate did not carry the *ermB* gene. Three isolates belonging to RT 014 (n = 1) and RT 017 (n = 2), which carried the *ermB* and *tetM* genes, were resistant to CLD, ERY and TET.

The remaining three isolates RT 014 (n = 1), RT 056 (n = 1) and RT 446 (n = 1) were susceptible to CLD, ERY and TET, and were not found to carry the *ermB* and the *tetM* genes. Interestingly, one isolate of the RT 039 harbored the *tetM* gene, but was susceptible to TET.

The two isolates of the RT 014, which belonged to two different STs, ST2 and ST14, also exhibited different antibiotic resistance phenotypes and genotypes; one isolate (RT 014/ST14) was resistant to CLD, ERY and TET, and carried the *ermB* and *tetM* genes; whereas the second (RT 014/ST2) was susceptible to these three antibiotics and did not carry the *ermB* and *tetM* genes. Of note, these two isolates were recovered from two different patients admitted to different wards of the same hospital.

**Discussion**

*C. difficile* has been identified as a leading nosocomial pathogen worldwide and the main causative agent of antibiotic-associated diarrhoea in humans [1]. Although *C. difficile* infections were generally regarded as primarily healthcare-associated, and community-acquired, *C. difficile* infections have now emerged as a significant public health concern [2]. Algeria is at the crossroads to Europe, Africa, and the Middle-East; and like in many developing countries, CDI is a largely neglected disease, and epidemiological data on *C. difficile* are scarce. There is, however, one previous study, based in two hospitals in one province [10]. Therefore, this study was conducted to investigate the prevalence and the molecular epidemiology of *C. difficile* over a wider geographic region, in five study sites in three provinces of Algeria. Interestingly, the prevalence of *C. difficile* estimated here (6%) was similar to the previous Algerian study (6.9%) [10].

The prevalence estimated in this study was comparable to those reported in certain African and Middle-Eastern countries such as Ghana (4.9%) [22], Tanzania (6.4% and 7.3%) [23,24], Zimbabwe (8.6%) [25], Iran (9%) [26,27], Qatar (7.9%) [28] and Saudi Arabia (8.4%) [29], and also falls within the range reported in a European multi-country surveillance study, from 4% to 39% [30] and in the United States from 6% to 48% [31].

Compared to the previous Algerian study, which reported the detection of only four ribotypes [10], our study revealed a relatively larger diversity of PCR ribotypes. The most prevalent RT were RT 085 and FR 248 (n = 3, 16.7%, each), followed by RT 014/RT 017/FR 111 (n = 2, 11.1%, each) and RT 005/RT 029/RT 039/RT 056/RT 446/ FR 247 (n = 1, 5.6% each). All isolates of the same RT belonged to the same ST, with the exception of RT 014, which was shared by two different STs, ST2 and ST14 (1 isolate each), which is in agreement with a previous study [32]. Among the above ribotypes, only RT 014 was previously reported in Algeria, but in a different hospital [10].

The ten *C. difficile* isolates were non toxigenic and belonged to ribotypes RT 039, RT 085, FR 111, FR 247 and FR 248; with RT 085 and FR 248 as the most prevalent (n = 3, 16.7%, each). The three isolates of RT 085 were detected in three different wards of the same hospital in the eastern province of Algeria (Batna), but were missing in the two other provinces; suggesting a possible distinct geographic distribution of this ribotype in Algeria. Although, the ribotype RT 085 was reported as more common in China [33], it was rarely reported in other countries.

It is also worth mentioning that all the three isolates belonging to the unclassified ribotype FR 248 were recovered from children, that were admitted to two different hospitals from two provinces (Ain Defla and Chlef), and as such, the possibility of an association of this ribotype with children is plausible.

The remaining non toxigenic ribotype isolated with a lesser frequency (n = 1), RT 039, was previously reported as most common in patients with cystic fibrosis in Western Australia [34], and was also detected in health care settings in Iran [35], and Kuwait [36]. Surprisingly, several isolates of RT 039 from Iran were found to carry the toxin genes [35]. In addition, isolates of RT 039 were also recovered from animal samples in the Netherlands [37] and Egypt [38].

Toxigenic isolates accounted for 44.4% (8/18) of the total and were shared between six ribotypes, 005, 014, 017, 029, 056 and 446; among which, those belonging to RTs 014 and 017 were the most frequent (n = 2, 11.1%, each). Isolates of the RT 014 were the most prevalent ribotype in many European countries, where it was reported as responsible for CDI outbreaks in humans, and also commonly associated with animals and different environments [37,39–42]. The RT 014 was also detected in several countries in the Middle-East, Iran [35], Lebanon [8] and Qatar [28]; whereas in the African continent it was reported only in Algeria [10] and South Africa [43].
The other most prevalent ribotype in this study, RT017, which is characterized by a deletion in the tcdA gene and the absence of binary toxin genes and, therefore, A-B+CDT- [44], is the predominant ribotype in Asia, and has also caused major outbreaks of CDIs in several countries around the world [45,46]. To date, the only African country that reported the ribotype RT 017 is South Africa [43,47], but it has not been detected so far in any of the Middle-eastern countries.

The toxigenic PCR-ribotype RT 029 was previously reported as one of the most frequent RTs among hospitalized patients in Iran [35], and was also isolated from humans in Egypt [48] as well as from humans and animals in Costa Rica [49].

The toxigenic isolate belonging to the PCR-ribotype RT 056 detected in this study, was commonly isolated from humans, cattle, vegetables and the environment in Australia [42]. Prior studies reported that RT 056 was frequently associated with complicated CDI in hospitalized patients in Europe [39,50]. The only report to date of this PCR-ribotype in Africa comes from Zimbabwe [25], whereas in the Middle-East it was reported in Qatar [28] and Kuwait [9,36].

Although the toxigenic RT 005 identified in this study is among the most common ribotypes in Europe [51], it was isolated with a low frequency in a study from Ghana [52], and has not been documented so far in the Middle-East.

Importantly, both this and the previous Algerian study failed to detect the hypervirulent ribotypes RT 027 or RT 078. It must be noted that the ribotype RT 027 was not reported in the African continent, whereas, in the Middle-East, it was detected, albeit with low frequency, in Iran (n = 14) [26,53,54], Saudi Arabia (n = 4) [55] and Qatar (n = 1) [28]. Similarly, there is very little data on the ribotype RT 078 in Africa and the Middle-East, except for two reports from Egypt (n = 6) and Kuwait (n = 9) [36,48].

It is worth mentioning that the detection of the toxigenic ribotypes RT014, RT017, RT029 and RT 056 in this study is important and interesting from an epidemiological point of view, given that these ribotypes were reported to be either responsible for CDIs in several countries around the world (RT017, RT 014 and RT 056) [50], or commonly associated with animals (RT 014, RT 029 and RT 056) [56], raising concerns about their potential zoonotic transmission.

Antibiotic susceptibility test results have shown that all 18 C. difficile isolates were susceptible to VAN and MTZ, the treatment of choice for CDI [57], as well as to MXF, a fourth generation fluoroquinolone [58], which is in line with the results of several other studies [59].

Thirteen MLSB-resistant isolates (72.2%, 13/18), carried the ermB gene, and were resistant to CLD and/or ERY; whereas two (11.1%, 2/18) MLSB-resistant isolates were ermB-negative but resistant to either CLD or ERY; suggesting that the MLSB resistance in these isolates might be conferred by other mechanisms; which is in agreement with previous studies [20,60]. We noticed that resistance to TET was always associated with co-resistance to CLD and ERY, as is the case for three isolates belonging to RT 014 (n = 1) and RT 017 (n = 2), which harboured both the tetM and the ermB gene. The other TET resistance genes investigated in this study (tetO, tetB[P], tetO/32/0, tet40, tetA[P]) were not detected in any of our 18 isolates. Surprisingly, one isolate, member of RT 039, was susceptible to TET despite carrying the tetM gene. Given that tetM is the predominant TET resistance genetic determinant in C. difficile, the exact mechanism behind this peculiar phenotype is unclear at this stage; it is possible, however, that the tetM gene in this isolate was inactive due to a mutation.

The high rate of resistance of RT 017 to many antimicrobial agents has been largely documented in several studies, and considered as a major contributing factor to the success and dissemination of this ribotype throughout the world [61,62].

There are a number of limitations within this study that needs to be highlighted; first, and most important, our study lacked clinical patient data; second, this is a study based on a small sample size of isolates; third, C. difficile isolates were collected in three geographical areas and five hospitals, which may limit the generalization of the C. difficile prevalence estimates to the whole country; fourth, lack of antibiotic susceptibility testing against other important antibiotics used for the treatment of CDI such as fidaxomicin and rifaximin. Clearly, larger studies, over wider geographical area and larger number of study sites are merited.

Conclusions

The present study revealed a moderate prevalence of CDI (6%), with a relatively high diversity of C. difficile isolates, some of which were toxigenic. All isolates were susceptible to VAN and MTZ; whereas a high proportion of the isolates showed resistance to CLD and/or ERY. Although well-known hypervirulent C. difficile strains such as RT 027 and RT 078 were not detected in this study, our findings highlight the significance of this pathogen in a sample of the Algerian
population, and therefore, an active surveillance of CDI is crucial in order to have a more generalized estimation of the burden of this disease in the country.

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Authors’ contributions
YB collected the specimens, and isolated, cultured and confirmed the tested isolates. YB, JC, RSZ and FB confirmed the tested isolates with mass spectrometry, carried out the toxin gene profiling and ribotyping; and performed the antibiotic resistance tests. KM performed the MLST and characterized the antibiotic resistance genes. DA helped in the culturing, identification of the isolates and interpretation of the results. SR was involved in the inclusion of one group of patients and provided their specimens. MEB performed the statistical analysis. YB wrote the draft manuscript. FB, KM, DA and MS reviewed the manuscript. MS conceived the study, supervised the research and revised the manuscript.

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