Molecular Detection of the Arr-2 Gene in *Escherichia coli* and *Klebsiella pneumoniae* Resistant to Rifampicin in Abidjan, Côte D'Ivoire

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors VG, NKG and JAD designed the study, wrote the protocol. Authors JMR, SMD and MD provided the material and equipment. Authors AAT, FK and MBO contributed to the writing and editing of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/40552

Original Research Article

Received 5th January 2018
Accepted 28th March 2018
Published 11th April 2018

ABSTRACT

**Aims:** The aim of this study was to demonstrate the presence of rifampicin resistance arr-2 gene in certain enterobacteria of clinical origin from various biological products.

**Place and Duration of Study:** National Reference Center for Antibiotics of Pasteur Institute of Côte d'Ivoire, and research unit on emerging tropical infectious diseases of Aix-Marseille University.

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between January to July 2017.

**Methodology:** The strains were isolated from different biological samples and identified by biochemical tests and MALDI-TOF MS mass spectrometry. The Antibiotic susceptibility testing was performed by diffusion on Mueller- Hinton (MH) agar.

**Results:** All strains were resistant to rifampicin (100%) and amoxicillin (100%), but sensitive to imipenem and colistin. Conventional PCR using specific primers detected the arr-2 gene in four strains, including 3 strains of *Klebsiella pneumoniae* and one strain of *Escherichia coli*. Molecular typing of the strains by MLST showed that the *E. coli* strain had ST 5 and the three strains of *K. pneumoniae* had ST 307, ST 309, ST 273 respectively.

**Conclusion:** This article is the first report that highlights the presence of the arr-2 gene in enterobacteria in Côte d’Ivoire.

**Keywords:** *Escherichia coli; Klebsiella pneumoniae; rifampicin; arr-2 gene; Abidjan.*

### 1. INTRODUCTION

Rifampicin is an antibiotic used in the treatment of tuberculosis [1], meningitis [2] and against Staphylococcus aureus infections [3,4]. It works by binding to the β-subunit of the RNA polymerase and inhibits the transition from initiation to elongation during transcription [5].

Resistance to rifampicin was mainly due to the mutations in the *rpoB* gene that encodes the β-subunit synthesis of bacterial RNA polymerase, resulting in decreased binding of R-RNA polymerase to rifampicin [6]. Presently, several others resistance mechanisms are identified, including efflux systems [7], glycosylation [8,9], phosphorylation [10] and inactivation of the antibiotic by ADP-ribosyltransferase (Arr) [11]. The first arr-1 resistance gene has been described in *Mycobacterium smegmatis* [12] and subsequently the arr-2 and arr-3 genes have been described, carried by class I integrons in gram-negative bacilli in Europe and Asia [13,14, 15]. In particular, the arr-2 gene is present on several transposons and integrons found in strains of *Pseudomonas aeruginosa* [16], *Escherichia coli* [17], *Klebsiella pneumoniae* [13] and *Acinetobacter baumannii* [18].

The objective of the present work was to highlight the presence of the arr-2 gene in rifampicin-resistant clinical enterobacteria isolated from Abidjan.

### 2. MATERIALS AND METHODS

#### 2.1 Bacterial Strains

The strains that were used in this study were 153 in number mainly of Enterobacteriaceae producing Extended-Spectrum Beta-Lactamase (ESBL) and those that did not. They were isolated from different biological samples, previously identified by biochemical tests and stored at the Center of Biological Resources (CeReB) of the Pasteur Institute of Côte d’Ivoire. Their identification was confirmed by MALDI-TOF MS (Bruker) mass spectrometry.

#### 2.2 Antibiotic Susceptibility Test

Antibiotic susceptibility was determined from young colonies grown on Mac Conkey agar (BioMérieux SA, France) for 24 hr. The antibiotics used to perform the antibiogram were: amoxicillin (25 μg), amoxicillin + clavulanic acid (20 μg + 10 μg), ticarcillin + clavulanic acid (75 μg + 10μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftriaxone (30 μg), aztreonam (30 μg), imipenem (10 μg), ertapenem (10 μg), amikacin (30 μg), gentamicin (15 μg), ciprofloxacin (5 μg), fosfomycin (50 μg), colistin (50 μg), cotrimoxazole (25 μg), rifampicin (30 μg). Mueller-Hinton agar growth medium was used for diffusion according to the method described by the Antiibiogram Committee of the French Society of Microbiology (CA-SFM, 2013).

#### 2.3 Total DNA Extraction

Extraction of the total DNA from the strains was performed from 24 h young colonies using the G2 buffer contained in the EZ1 DNA Tissue Kit (QIAGEN). Using a vortex, a colony of the strain was homogenized in 200 mL of buffer G2. The homogenate was then introduced into the EZ1 automated for extraction. The DNA thus extracted was stored at -20°C.

#### 2.4 Gene Detection by Conventional PCR

Conventional PCR was performed for detection of arr-2 using the following primers: arr-2_Foward_AATTACAAGCGGTGCAAGGA and arr-2_Reverse__TTCAATGACGTGTAACCACG. The reaction
was carried out in a reaction volume of 25 μL consisted of 12.5 μL of Master Mix (Quantitect Probe PCR master mix, Qiagen), 1 μL of the primer mixture (Eurogentec), 6.5 μL of UP water and 5 μL of total DNA. The amplification consisted of an initial denaturation step of the double-stranded DNA for 15 min at 95°C. This step was followed by 35 amplification cycles comprising denaturation at 94°C for 1 min, hybridization at 55°C for 50 sec, elongation at 72°C for 2 min and final elongation of 7 min at 72°C.

2.5 Visualization of Amplified Genes

The PCR products were analysed using 1.5% agarose gel electrophoresis prepared with 0.5% Tris-Borate-EDTA (TBE). The BenchTop pGEM DNA Markers Cat.# G7521 (Promega, France) molecular weight marker were used and the migration lasted for 20 minutes at a voltage of 135 V. The visualization of the DNA bands were done on a transilluminator.

2.6 PCR, Sequencing and Molecular Typing by MLST of Resistant Strains

To determine the typical sequences of the resistant strains, a conventional PCR using specific primers of the house-keeping genes per species was carried out. The functions of these genes and the sequences of the primers were summarized in Tables 1 and 2. The purified PCR products were sequenced using the BigDye® kit (Invitrogen, Life Technologies, North America) following the manufacturer’s user’s guide, in the automated ABI PRISM 3730xl Genetic Analyzer. Sequence analysis was performed on the MLST websites of the Pasteur Institut of Paris and MLST Warwick in order to assign a standard sequence number according to the allelic profiles of the different strains.

3. RESULTS

3.1 Antibiotics Susceptibility

All strains showed resistance 100% to rifampicin. For the resistance to amoxicillin alone, it was 100%, while the resistance rate to amoxicillin/clavulanic acid was 99.3%. The susceptibility test showed high resistance to third-generation cephalosporins, cefotaxime (85.6%), ceftriaxone (81%) and cefoxitin (80.4%) respectively. The trimethoprim-sulfamethoxazole and fosfomycin resistance levels were 86.3% and 16.3%, respectively. The aminoglycoside resistance rate was 80.4% and 81% of the strains resistant to ciprofloxacin. However, all strains were sensitive to imipenem and colistin.

3.2 Distribution of Rifampicin Resistance Genes and MLST

The arr2 gene was detected in four strains, 1 E. coli and 3 K. pneumoniae (Fig. 1). Table 3 presents the epidemiological profile of the 4 strains carrying the arr-2 gene. Fig. 2 shows the visualization of the 7 house-keeping genes of the E. coli and K. pneumoniae species after electrophoresis. Strain typing by MLST analysis showed that E. coli strain had ST 5 and all three strains of K. pneumoniae had ST 273, ST 307 and ST 309 respectively.

Table 1. Primers used in MLST for E. coli

<table>
<thead>
<tr>
<th>Gene (function)</th>
<th>Primer sequence (5' → 3')</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>adk (adenylate kinase)</td>
<td>ATTCTGCTTGCGCGCTCCGG (F)</td>
<td>583</td>
</tr>
<tr>
<td></td>
<td>CCGTCATCTTCCCGGTATT (R)</td>
<td></td>
</tr>
<tr>
<td>fumC (fumarate hydratase)</td>
<td>TCACAGTCGCCACGGCTTC (F)</td>
<td>806</td>
</tr>
<tr>
<td></td>
<td>GTAGCGGAGAAAGATTCA (R)</td>
<td></td>
</tr>
<tr>
<td>icd (isocitrate/isopropylmalate déshydrogénase)</td>
<td>ATGGAAGGATAGATGGTTCGGCGACA (F)</td>
<td>878</td>
</tr>
<tr>
<td></td>
<td>GGACGCAGCAAGATTCA (R)</td>
<td></td>
</tr>
<tr>
<td>purA (adenylosuccinate déshydrogénase)</td>
<td>CGCGCTGATGAAAGAGATGA (F)</td>
<td>861</td>
</tr>
<tr>
<td></td>
<td>CATACGTAAGCCACGCAGA (R)</td>
<td></td>
</tr>
<tr>
<td>gyrB (DNA gyrase)</td>
<td>TCGGCGACACGGATGACGCC (F)</td>
<td>911</td>
</tr>
<tr>
<td></td>
<td>ATCGGGTACCCGCAAGCTC (R)</td>
<td></td>
</tr>
<tr>
<td>recA (ATP/GTP binding motif)</td>
<td>CGCACTCGGCTCACTCTCTGAC (F)</td>
<td>780</td>
</tr>
<tr>
<td></td>
<td>TCGTCGAAATCAGGTACCCGGA (R)</td>
<td></td>
</tr>
<tr>
<td>mdh (malate déshydrogénase)</td>
<td>ATGAACTCGAAGTACCCGGAAGATCG (F)</td>
<td>932</td>
</tr>
<tr>
<td></td>
<td>TTAACGAACTCCTGCGCAGA (R)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Primers used in MLST for \textit{K. pneumoniae}

<table>
<thead>
<tr>
<th>Gene (function)</th>
<th>Primer sequence (5'→3')</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
</table>
| \textit{rpoB} (beta-subunit of RNA polymerase) | GTTTTCCCCAGTCACGACGTTGGTAGGCGAAATGGCGWAGAACC (F)  
TTGTGACGGATAACAATTTTCGAGTCTTGAAGTTACC (R) | 501                |
| \textit{gapA} (Glyceraldehyde-3-phosphate dehydrogenase) | GTTTTCCCCAGTCACGACGTTGGTAGAATATGACTCCACTCAGGG (F)  
TTGTGACGGATAACAATTTCCTCGGAAGCGGCTTTGATGCTT (R) | 450                |
| \textit{mdh} (malate dehydrogenase) | GTTTTCCCCAGTCACGACGTTGGACCCACTCGCTTACGTTACG (F)  
TTGTGACGGATAACAATTTTCCGAGTGTTACGTAACCG (R) | 477                |
| \textit{pgi} (phosphoglucose isomerase) | GTTTTCCCCAGTCACGACGTTGGACGTTCTACGCTTACCGTCC (F)  
TTGTGACGGATAACAATTTTCCGAGTGTTACGTAACCG (R) | 432                |
| \textit{phoE} (phosphorine E) | GTTTTCCCCAGTCACGACGTTGGACTACCGTCCGTTACGTAACCG (F)  
TTGTGACGGATAACAATTTTCCGAGTGTTACGTAACCG (R) | 420                |
| \textit{infB} (translation initiation factor 2) | GTTTTCCCCAGTCACGACGTTGGACTACCGTCCGTTACGTAACCG (F)  
TTGTGACGGATAACAATTTTCCGAGTGTTACGTAACCG (R) | 318                |
| \textit{tonB} (periplasmic energy transducer) | GTTTTCCCCAGTCACGACGTTGGACTACCGTCCGTTACGTAACCG (F)  
TTGTGACGGATAACAATTTTCCGAGTGTTACGTAACCG (R) | 414                |

Table 3. Epidemiologic profile of the 4 strains carrying the \textit{arr-2} gene

<table>
<thead>
<tr>
<th>Strains</th>
<th>Services</th>
<th>Biological sample</th>
<th>Typical sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. coli} 725YO/15</td>
<td>Neurology</td>
<td>Blood</td>
<td>ST 5</td>
</tr>
<tr>
<td>\textit{K. pneumoniae} 868Y/13</td>
<td>Pediatrics</td>
<td>Suppuration</td>
<td>ST 307</td>
</tr>
<tr>
<td>\textit{K. pneumoniae} 1141Y/15</td>
<td>Neonatal maternity</td>
<td>Urine</td>
<td>ST 309</td>
</tr>
<tr>
<td>\textit{K. pneumoniae} 654UB/15</td>
<td>Pneumonology</td>
<td>Sputum</td>
<td>ST 273</td>
</tr>
</tbody>
</table>
Fig. 1. Electrophoresis of arr2 genes
M: Molecular weight marker
T-: negative control
S1: K. Pneumoniae 868Y/13
S2: K. Pneumoniae 1141Y/15
S3: E. coli 725YO/15
S4: K. Pneumoniae 654UB/15

Fig. 2. Visualization of 7 house-keeping genes of E. coli and K. pneumoniae
E.c1: E. coli 725YO/15
K.pn1: K. Pneumoniae 868Y/13
K.pn2: K. Pneumoniae 1141Y/15;
K.pn3: K. Pneumoniae 654UB/15

4. DISCUSSION

Resistance to rifampicin is a serious public health problem [19] because it is the first-line drug for the treatment of tuberculosis [20] and the prevention of meningitis [2]. Rifampicin resistance induced by the arr-2 gene has been described in several bacterial strains including P. aeruginosa [16], E. coli [17], K. pneumoniae [13] and A. baumannii [18]. Detection of the arr-2 gene in strains of K. pneumoniae and E. coli is the first of its kind in Côte d'Ivoire could explain rifampicin resistance observed in strains. However, the presence of this gene only in four strains while the resistance rate was 100%, suggests that strains use others mechanisms of resistance to rifampicin such as efflux systems [7], glycosylation [8,9], phosphorylation [10] or
resistance may be due to other resistance genes such as arr-3 [13].

Moreover, the typing of strains with the arr-2 gene by MLST analysis showed that an E. coli strain had ST 5. This sequence (ST 5) was found in an E. coli strain isolated from stool from a patient in Douala, Cameroon, which also carried the β-lactam resistance genes blaNDM-1 and blaOXA-1. The patient had been hospitalized for a month in Douala for an inflammatory syndrome associated with renal failure before his transfer to Paris [21].

Also, the three strains of K. pneumoniae carrying the arr-2 gene had ST 273, ST 307, and ST 309, respectively. These different standard sequences have also been obtained elsewhere in the world. The type 273 sequence was found in a strain of K. pneumoniae, isolated from a patient with pneumonia in the Philippines, which carried the blaNDM-7 gene [22]. K. pneumoniae ST 273 has been reported in Italy, Norway, Russia and the United Kingdom. These studies reported that ST273 isolates carried various carbapenemase genes including blaKPC, blaNDM-1 and blaVIM, and ST 273 is known to have the epidemic potential [23,24].

Regarding the ST 307 sequence, work done in Italy showed that strains of K. pneumoniae isolated from three Italian hospital centers and which carried the KPC-2, KPC-3 and blaCTXM-15 genes had ST 307 [25]. ST 307 was first described in 2008 in the multi-locus sequence typing database (MLST) (an unpublished isolate) and has since been described in 2013 in the United States [26]. It was initially associated with CTX-M-15 expanded spectrum β-lactamase production. The acquisition of a KPC enzyme is posterior to that of CTX-M-15, as Kp ST 307 CTX-M-15 producer was previously reported at a high rate in Italy, Korea, Pakistan, Morocco and in domestic animals from Japan [27-28].

As for the type 309 sequence, it was found in strains of K. pneumoniae isolated from hospitalized patients in northern China [29].

4. CONCLUSION

This study has highlighted the high resistance to rifampicin in enterobacteria of clinical origin in Côte d’Ivoire. The arr-2 resistance gene was detected in only four strains, suggesting that there would be other mechanisms of rifampicin resistance used by the strains that should be explored. Multilocus sequence typing (MLST) has made it possible to genetically characterize resistant strains and highlight the circulation of various clones around the world.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24106