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Letter to the Editor

Salmonella Kentucky: Antimicrobial resistance and molecular analysis of clinical, animal and environment isolates, Morocco

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Dear Editor,

Salmonella infection is a major public health problem worldwide. Various animals (especially poultry, pigs, cattle, and reptiles) are reservoirs for *Salmonella* species, and humans generally get infected by eating undercooked or contaminated food [1].

In Egypt, during the period 2002-2005 *Salmonella* Kentucky resistance to ciprofloxacin was a major health problem and from there it spread throughout Africa and Middle East [2]. In Morocco, *Salmonella* Kentucky ciprofloxacin-resistance was first identified in an eight-month old child who was admitted at the paediatric department of the University Hospital Centre Ibn-Rochd in Casablanca with an acute febrile diarrheal [3]. It is estimated that every year 94 million persons are affected by nontyphoidal salmonellosis (NTS) infections worldwide, with about 155,000 deaths [4]. The drugs of choice are quinolones for adults and third-generation cephalosporins for children [4]. In Morocco, *Salmonella, Staphylococcus aureus* and *Clostridium perfringens* were reported to be responsible for respectively, 42.8%, 37% and 1.7% of food poisoning cases in humans [5]. Of the 1,577 cases of epidemic food poisoning reported annually in Morocco, *Salmonella* was confirmed in 6,1%(96 cases) and suspected in 16,4%(259 cases) [6]. In this study, we provide the first comprehensive report on the dissemination and characteristics of the *Salmonella* Kentucky isolates found in turkey, laying hens farms and human food chain in Morocco.

The study

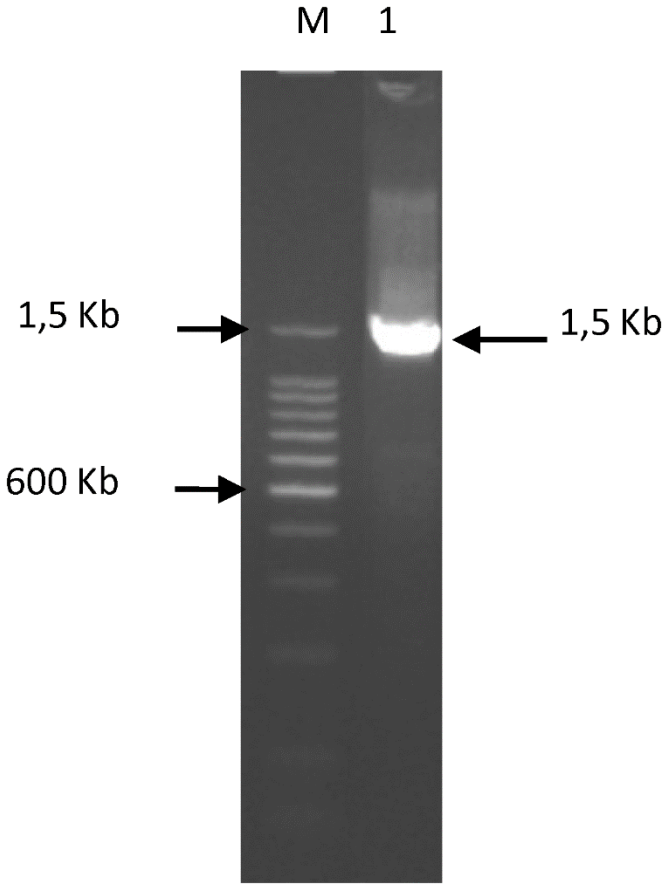
A total of 434 *Salmonella* enterica isolates, were serotyped between 2005 and 2015at the Food Microbiology Laboratory at Pasteur Institute of Morocco. Regardless of their origin, the vast majority [(122/434) 28,1%] of the isolates was found to belong to *Salmonella* Kentucky serotype.

The source of the 122 isolated *Salmonella* Kentucky was as follow: 15,6% (19/122) were isolated from humans in Casablanca, 29,5% (36/122) from shellfish in the northwest Moroccan Mediterranean coast, 11,5% (14/122) from raw minced meat of turkey in Casablanca, 16,4% (20/122) from laying hens farms [droppings (n = 12), sample dust (n = 4), food (n = 3) and water (n = 1)] in Souss-Massa-Draa Rabat-Sale-Zemmour-Zaer and Casablanca, 7,4% (9/122) from turkey carcasses and giblets in Meknes,6,5% (8/122) fromice cream in Casablanca, 4,1% (5/122)from raw minced meat of beef in Tetouan, 2,5% (3/122) from

milk in Fes and 6,5% (8/122) were from an unknown origin.

Antibiotic susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar (MHA) and results were interpreted according to the EUCAST breakpoints (Committee of the French Society for Microbiology (CA-SFM)). Minimal inhibitory concentration was determined by the E-Test method (AB Bio-disk, Solna, Sweden).Serotype was performed using the slide agglutination test with specific anti-sera raised against "0" and "H" antigens of *Salmonella* (BioRad, Marnes-La-Coquette, France) [7]. Our data showed that 902% (110/122) isolated S. Kentucky strains showed a high resistance level to ciprofloxacin (MIC 4-16 µg/mL) [1].Furthermore, these strains were multiresistant to amoxicillin, tetracycline, chloramphenicol, sulfonamides, gentamicin, streptomycin and nalidixic acid. However, 9,8% (12/122) strains were susceptible to all the tested antibiotics.

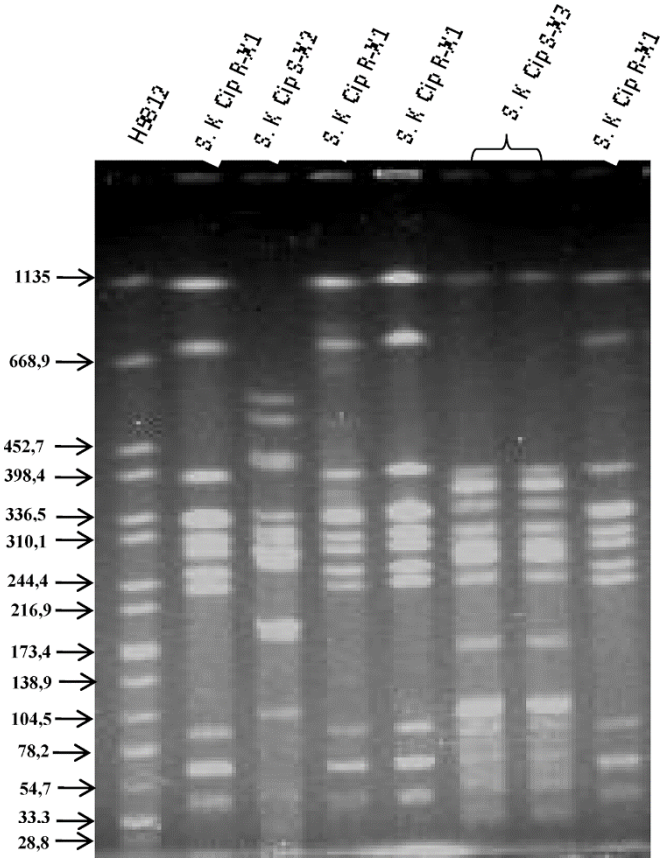
Figure 1. Agarose gel (0,75%) of PCR products for analysis of class 1 integron.



*M: Molecular weight marker 100 bp DNA Ladder; Column 1 represent integron class1 gene (1500 bases pairs).

To identify the mutations responsible of resistance, ciprofloxacin, the quinolone resistance–determining regions (QRDRs) of GyrA, and ParC, were amplified by PCR and sequenced as described previously [8]. Our results showed that all ciprofloxacin-resistant S. Kentucky harbored diverse double mutations in GyrA gene: Ser83Phe and Asp80Asn.An exception was the VIM-2 producing Kentucky isolate from human, which had Asp87Tyr [9]. In contrast, these isolates showed the same mutations in the ParC gene: Ser80Ile and Thr57Ser substitution at the topoisomerase protein. In addition, all these strains were negative for the plasmid mediated quinolone resistance conferring gene qnrA,B,S. Furthermore, *Salmonella* Kentucky strains resistant to amoxicillin, tetracycline, gentamicin, streptomycin, chloramphenicol and sulfonamides were found harbor the integron class1 gene (1,5Kb) (Figure 1). It is also noteworthy that, according to our previous publication, ciprofloxacin-resistant S. Kentucky strains in Morocco were found to have the ability to acquire

Figure 2. Representatives of *XbaI*-PFGE profiles obtained among S. Kentucky isolates from human, animal and environment.



*H9812: *Salmonella*Braenderup used as a marker - S.K. Cip R-X1: *XbaI* profile from S. Kentucky ciprofloxacin-resistant - S.K. Cip S-X2: *XbaI* profile from S. Kentucky ciprofloxacin-susceptible.

new resistance to carbapenemases producer (OXA-48 and VIM-2) [9].

For molecular typing, we performed PulseNet standard pulsed-field gel electrophoresis (PFGE) of *XbaI*-digested chromosomal DNA on all S. Kentucky isolates. Two new pulse-field profiles (X2, X3) were identified in ciprofloxacin sensitive S. Kentucky strains, while one pulse-field profile (X1) was identified in ciprofloxacin-resistant S. Kentucky (Figure 2).

Conclusion

In the present study, the implementation of these highly resistant bacterial strains in turkey and laying hens farms visited may promote their release in other ecosystems, particularly the marine coastline by waste water. The spread of these resistant bacteria (especially ciprofloxacin-resistant S. Kentucky) needs to be stopped and timely and transparent information is needed for the doses of antibiotics used in agriculture and on resistant bacteria in foods. Measures to monitor and limit the spread of these strains in Morocco should be implemented.

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