

Bacterial Resistance of *Enterobacterae* isolates in Western Algeria

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ABSTRACT

Infections due to Enterobacteria are responsible for significant losses in the poultry industry and are the most frequent causes of carcass rejection at the slaughterhouse. Antibiotics can contribute to reduce bacterial infections. Their use has increased in recent years.

In western Algeria, 150 strains of commensal enterobacteriaceae were isolated (including 101 *Escherichia coli*, 27 *Proteus mirabilis*, 13 *Enterobacter cloacae*, 9 *Klebsiella pneumoniae*) from different origins in western Algeria. Serotyping of 51 *Escherichia coli* strains demonstrated that 28% of their serogroups belong to the serogroups of Avian Pathogenic *Escherichia coli* (APEC): O1 (28%), O78 (14%), O2 (6%), O78 and 52% were non-typable.

In vitro susceptibility to antimicrobials was determined by disc diffusion test. The majority of isolates was resistant to betalactam, quinolone, tetracycline, trimethoprim sulfamethoxazole. *Escherichia coli* strains present the high levels of resistance: nalidixic acid 84%, flumequin 94%, enrofloxacin 86%, Tetracycline 92%, Trimethoprim/sulfamethoxazol 91%, Amoxicillin 92%, cefalotin 80%. *Proteus mirabilis*, *Enterobacter cloacae* and *Klebsiella pneumonia* (respectively) showed resistance to nalidixic acid (81, 77, 100%), flumequin (81, 84, 100%), enrofloxacin (77, 53, 77%), tetracycline (100, 84, 77%), trimethoprim sulfamethoxazole (74, 69, 77%), amoxicillin (62, 77, 100%), ceftiofur (44, 61, 55%).

All isolates showed low resistance to colistine, furane, Amoxicillin/clavulanic acid, gentamycin, chloramphenicol and neomycin, with the exception of the *Proteus* isolates which were...

All isolates were resistant to at least two antibiotics, 63% to 6 antibiotics and 4% to 11 antibiotics.

For the PCR tests, No antimicrobial resistance quinolone gene was detected after testing 20 *E. coli* isolates.

The high rate of antimicrobial resistance in bacterial isolates may have major implications for human and animal health with adverse economic implications.

KEY WORDS: *E. coli*, antibiotic, antibiogram, chicken, PCR.

1. INTRODUCTION

Enterobacteria infections are responsible for of great economic losses in the poultry industry. Despite increased resistance, prophylaxis and antibiotherapy remain the sole mean to fight these bacteria.

Bacterial resistance to the antimicrobial agents is a problem of increasing importance in medical practice [1]. The scattering of resistant bacteria has led to considerable increase of mortality and morbidity as well as the cost of treatments [2]. High concentrations of these microbes in the digestive tract favors exchange and scattering of resistance genes [3, 4].

The present work was conducted to estimate the antimicrobial resistance of Enterbacteria isolates from chicken in western Algeria.

2. MATERIALS AND METHODS

2.1 Sampling Site and Procedure

The study was conducted in western Algeria (Mostaganem, Mascara, Tiaret, Tlemcen, Ain Temouchent and Sidi Bel Abess) from September 2010 to July 2015. Samples for isolation included surfaces (soil swabbing, walls and ceiling), eggs and droppings.

The samples were directly seeded on agar bromocresol purple (BCP) Mc Conkey Sorbitol and incubated for 24 h at 37 °C.

The macroscopic and microscopic identification (bacilli Gram with peritrichous cilia), the biochemical tests and the analytical profile index 20E system (Biomerieux, France) were performed according to Quinn [5].

2.2 Serotyping

Fifty *E. coli* isolates, from Tlemcen, Ain Temouchent and Sidi Bel Abess region were serotyped by agglutination test using specific antiserum raised against O1:K1, O2:K1, and O78 antigens (Biovac, France) according to Finazzi *et al.* [6].

2.3 Antibiogram:

Antibiotic sensitivity was determined by disc diffusion method [7] on solid Mueller-Hinton medium (Pasteur institute, Algeria) according to the guidelines of the CLSI [8].

Standard paper disks containing antibiotics used in Algeria (table 1) were laid on the medium. The plates were incubated for 24 h at 37 °C and the diameter of inhibition zones were interpreted by referring to the reading table of *Enterobacteria* as recommended by the Antibiogram Committee of the French Society for Microbiology [9].

Table 1 : Antibiotic discs

Famille	Antibiotic + Disc charge (µg)	Sigle	Provenance
Betalactam	Amoxicillin (10)	AMX	Bioanalyse, France
	Amoxicillin/clavulanic acid (20+30)	AMC	Bioanalyse, France
Cephalosporines	Ceftiofur (30)	EFT	Oxoid, Angleterre
	Cefalotin (30)	KF	Oxoid, Angleterre
Aminosides	Neomycin (30)	N	Bio-rad, France
	Gentamycin (10)	CN	Bioanalyse, France
Sulfamides	Trimethoprim/sulfamethoxazol (1.25)	SXT	Bioanalyse, France
Tetracyclines	Tetracycline (30)	TE	Bio-rad, France
	Nalidixic acid (30)	NA	Bio-rad, France
Quinolones	Flumequin (30)	UB	Bio-ra, France
	Enrofloxacin (5)	ENR	Bioanalyse, France
Polypeptides	Colistin (10)	CT	Oxoid, Angleterre
Furanes	Nitrofurantoin (300)	FT	Himedia, Inde
Phenicols	Chloramphinicol (30)	C	Bioanalyse, France

2.4 Detection of antibiotic resistant genes

Polymerase chain reaction multiplex with specific oligonucleotide primers were used to test for the occurrence of antibiotic resistant genes in 20 *E. coli* confirmed isolates that showed resistance to nalidixic acid, flumequin and enrofloxacin.

DNA of quinolone-resistant strains of *E. coli* was extracted by boiling method as per the guidelines [10].

PCR amplification of *qnr* genes (*qnrA*, *qnrB*, *qnrS*) were performed with the specific primers. PCR products were analyzed by electrophoresis in a 1 % agarose gel, containing ethidium bromide (1 µg/ml) buffered with Tris-borate-EDTA (0.1 %). PCR products were separated by electrophoresis on agarose gel (1 %).

3. RESULTS AND DISCUSSION:

In western Algeria, 150 strains of Enterobacteriaceae were isolated according to usual bacteriological procedures. *Escherichia coli* represented 67.33 % of these strains (N = 101) and *Proteus* 18 % (N = 27) (table 2).

Table 2: Distribution according to origin

	Eggs, Droppings, Surface Nb (%)	Broilers Nb (%)	Chickens Nb (%)	Total Nb (%)
<i>E. coli</i>	20 (13.33)	63(42)	17 (11.33)	101 (67.33)
<i>Proteus</i>	09 (06)	09 (06)	09 (6)	27 (18)
<i>Enterobacter</i>	05 (3.33)	02 (1.33)	06 (4)	13 (8.66)
<i>Klebsiella</i>	05 (3.33)	03 (02)	02 (1.33)	09 (06)
Total	39 (26)	77 (51.33)	34 (22.66)	150 (100)

E. coli was dominant (67%) followed by *Proteus* (18%), revealing that *E. coli* was the most widespread bacterium in the poultry rearing in accord with Ezekiel *et al.* (11) and Santos *et al.* (12) who reported same dominance with level of 80 % and 44 % respectively in poultry.

Moreover, Enterobacteria are frequently isolated in bacteriology laboratories, among them *E. coli* is the most most frequent species found [4; 13].

3.1 Serotyping

Among isolates 28 %, 6 % and 14 % belonged to O1, O2 and O78 pathogenic to poultry, respectively. However, 52 % of isolates were non- typables due to their serological diversity or are other serotype; O35, O88, O136. In contrast, Seifi *et al.* (14) found that O78 and O1 were the predominant serotypes.

3. 2 Antibiogram

Bacterial resistance was noted to every tested antibiotic (except *Klebsiella* to gentamycin) (table 3).

Table 3: Antimicrobial resistance frequencies (%)

Strain	NA	UB	ENR	TE	SXT	AMC	AMX	KF	EFT	CT	N	CN	FT	C
<i>E. coli</i>	84*	94*	86	92	91	17	92*	80*	41	21	27*	03	16	05
<i>Proteus</i>	81	81	77	100	74	85	62	03	44	74	62	14	96	62
<i>Enterobacter</i>	77	84	53	84	69	15	77	15	61	23	15	07	15	15
<i>Klebsiella</i>	100	100	77	88	77	22	100	22	55	11	22	00	55	22

NA: Nalidixic acid; UB : Flumequin; ENR : Enrofloxacin; TE : Tetracycline; SXT : Trimethoprim/sulfamethoxazol; AMC : Amoxicillin/clavulanic acid; AMX : Amoxicillin; KF : Cefalotin; EFT : Cefitofur; CT : Colistin; N : Neomycin; CN : Gentamycin; FT : Nitrofurantoin; C : Chloramphenicol.

* Microbial resistance assessed on 51 isolates

All isolates showed low resistance to colistine, furane, Amoxicillin/clavulanic acid, gentamycin, chloramphenicol and neomycin, with the exception of the *Proteus* isolates which were more sensible to gentamycin

Although chloramphenicol and gentamycin are prohibited in veterinary medicine in Algeria, the low resistance observed suggest either an illicit but uncommon use (because they are often cheaper and more accessible to the majority of population). Alternatively, they could be spontaneous mutants resistant to these antibiotics [15].

These frequencies seem high and allow dividing antibiotics into two groups; the first group includes antibiotics for which a high level of resistance is noticed:

- To *E. coli*, flumequin (94%), Tetracycline and Amoxicillin (92%), Trimethoprim/Sulfamethoxazole (91%), Enrofloxacin (86%), cefalotine (80).
- To *Proteus* : tetracycline (100%), furanes (96%), Amoxicillin (85%), nalidixic acid and flumequin (81%), Enrofloxacin (77%), Trimethoprim/Sulfamethoxazole and colistine (74%),
- To *Enterobacter* : flumequin and Tetracycline (84%), nalidixic acide and Amoxicillin (77%), Trimethoprim/Sulfamethoxazole (69%),
- And finally to *Klebsiella* : 100% resistant to nalidixic acid, flumequin and Amoxicillin, Tetracycline 88% and 77% to Enrofloxacin and Trimethoprim/Sulfamethoxazole. *Klebsiella* was sensible to gentamycin.

The second group comprises antibiotics with low resistance:

- To *E. coli*, amoxicillin/clavulinic acid, colistin gentamycin, nitrofurantoin and chloramphenicol.
- *Proteus*; cefalotin and neomycin
- *Enterobacter*; amoxicillin/clavulinic, cefalotin, neomycin, gentamycin, nitrofurantoin, chloramphenicol
- *Klebsiella* : colistin and gentamycin.

Gentamycin and chloramphenicol are forbidden in Algeria; the observed resistance can be explained by the illicit use of antibiotics that leads these bacteria to develop a resistance or a persistence of an older one.

Compared to other works in the same region, the rate of antimicrobial resistance of *E. coli* in the current timeframe seems to reveal an increased development of resistance for some antibiotics. Indeed, for three antibiotics, tetracycline, enrofloxacin and trimethoprim/tulfaméthazone, it passed respectively from 82%, 14% and 42% [16] to 87%, 45% and 70% [17] than to 90.4 %, 69.3 % and 70.2% in 2010 [18] to reach exceptional rates of 92 %, 86 % and 91% in this study.

Our results are higher than those reported in Eastern Algeria in 2014 for trimethoprim-sulfmethoxazole and enrofloxacin (82%) but resistance to tetracycline is reported to be even higher (100%) [19].

Likewise, the antibiotic resistance to amoxicillin, flumequin, nalidixic acid, amoxicillin and cefalotin is also very high. This phenomenon can be explained by the greater use of these antibiotics as well as diversity of the mechanisms of resistance of bacteria.

Resistance to colistin passed from 3 %, 5.5 %, 13 %, Hammoudi [16], Messai [19] and Benameur et al. [18] respectively to 21% in this study. This low resistance can be explained by the moderate use of this treatment on one hand and the fact that chromosomal mutations causing the resistance in colistin are rare [20].

As regards *Proteus*, high resistance was recorded especially for tetracycline (100%) in agreement with Nahar et al. (21) who reported a level of 94.4% in Bangladesh. The very high rates of resistance of *Proteus* strains to tetracycline, nitrofurantoines and colistine is possibly due to the natural resistance.

There are many studies on antimicrobial sensitivity on *Proteus* in humans but in poultry, no data were available [22]. Bacterial resistance was higher for quinolones (nalidixic acid and flumequin (81%), enrofloxacin (77%). These results are lower to those (93%) obtained by Nemati [22] in Iran but higher to those reported in Bangladesh [21] and can also be explained by the high level of utilization of these antibiotics widely available on the Algerian market.

At the Mediterranean level, the rates of resistance recorded in our study remain high compare to those registered in Morocco; 88% to amoxicillin, 67% to trimethoprim/sulfamethoxazol and 76% to enrofloxacin [23].

As was the case for trimethoprim/sulfamethoxazole (74%), antibiotic widely used in treatment of salmonellosis and during the coccidiosis what can be at the origin of a transfer of resistance.

These differences can be connected with difference in use frequency of these antibiotics to treat or prevent the diseases.

These microbial resistance rates, reflecting important use of these drugs in the poultry breeding, are worthy of note.

A considerable resistance was observed to Beta-lactams (amoxicillin/clavulanic acid (85%) and amoxicillin (62 %), widely superior to that (16%) obtained by Nahar *et al.* [21]. Emergence of resistance against β -lactam antibiotic is disturbing, since β -lactams are often remain the typical choice by the clinicians in treating a wide range of infections caused by *Proteus* spp. [24].

High levels of resistances were observed to quinolones (nalidixic acid 77% and 100 %, flumequin 84% and 100%), enrofloxacin (53% and 77%), tetracyclines (84%, 88%), trimethoprim/sulfamethoxazole (69% and 77%) in *Enterobacter* and *Klebsiella* respectively. These are all treatments that have been in routine for treating chicken against bacterial diseases.

The rates are less important to enrofloxacin than other quinolones; it is new generation that leads gradual emergence of the antibiotic resistance. More and more scientific evidence shows that these resistant bacteria, including pathogens, can be transferred to humans through the food chain [25].

Due to natural resistance, all isolates were resistant to amoxicillin. Less important resistances were observed to ceftiofur (61%, 55%) in *Enterobacter* and *Klebsiella* respectively. As well as low resistances to colistin cause moderate use and for chloramphenicol, gentamycin and furanes, molecules that are not used in veterinary medicine.

All strains of Enterobacteria isolated had at least two resistances towards these antibiotics, 95 % to at least 4 antibiotics and 4 % to 11 antibiotics (table 4).

Table 4: Strains of *Enterobacterea* multiresistant

Nb antibiotics	< 2	2	3	4	5	6	7	8	9	10	11
%	00	1.33	3.33	18	14	11.33	10	15.33	15.33	7.33	4

These results are close to those reported in Senegal where all strains were resistant to at least one antibiotic [26] and in Nigeria to at least three antibiotics [27]. The use of antibiotics could constitute a significant factor in the appearance of emergence selection and dissemination of resistant bacteria in veterinary medicine.

Multiresistance is probably due to the self-medication by breeders and alternating molecules before the first treatment produces results. There is no importance attached to the processing delay. Indeed, numerous antibiotics are administrated often concomitantly for prophylaxis or infections. This indicates that the abusive and indiscriminate use of antibiotic is probably the genesis of the high incidence of antibiotic resistance and multiresistances.

3.3 Genes of resistance

No quinolones antibiotic resistance genes were revealed testing 20 *E. coli* isolates. This can be explained by the fact that used primers could not allow screening all variants described so far and the low number of isolates examined [24]. In addition, other genes of plasmidic resistance in quinolones are recently described *qnr* C and *qnr* D and which are not amplifiable by these primers [28; 29].

Honoré *et al.* [30] reports prevalence of *qnr*-positive strains was globally of 0.7 % to 1.8 % among ESBL strains and of 0 % in quinolones resistant no-ESBL strains.

In 1998, a new mechanism of resistance in quinolones was described in a North American strain of *K. pneumoniae* [31]. Remarkably, this resistance is plasmidic i. e. transferable from an enterobacteria strain in another one. This localization on a DNA mobile element, explain the association of these genes of *qnr* type to genes coding for resistances to other families of antibiotics. Indeed, a wild-type *E. coli* strain carrying pMG252 plated onto agar containing nalidixic acid or ciprofloxacin was 100 times more likely to give rise to spontaneous resistant mutants than was a plasmid-free strain [32]. Moreover, it was demonstrated the *qnr* genes confer moderate levels of quinolone resistance, but can facilitate the selection of mutants showing high-level resistance [33].

Having been identified in the United States, this mechanism of resistance was found in many more North American strains [34]; several *E. coli* strains in China [28] and recently in *E. coli* strains from South Korea [35].

4. Conclusion

Enterobacteriaceae and specially *Escherichia coli* are commonly used as the indicator bacterium for the surveillance and monitoring of the emergence of antimicrobial resistance. Findings of our study show high levels of resistance, more importantly, emergence of resistance against a newer generations of antimicrobials (enrofloxacin) and multidrug resistance, is a call to attention.

We postulate many factors, such as, breeder's automedication, antibiotic switching before the first antibiotic has had a chance to produce results, and the heavy use of antibiotics without prior antibiogram test, in different ages in poultry houses.

The alarming rates of individual and multiple antimicrobial resistances in bacterial isolates from chickens may have major implications for human and animal health with adverse economic implications. It constitutes a public health hazard because of the possible transmission of these potential pathogens to humans through contact and consumption of contaminated food substances. This can lead to significant spread of the antibiotic-resistant bacteria; medicines used to treat human diseases will become less effective, and this is a very significant threat to public health.

Our finding demands stringent surveillance system to be developed in Algeria for antimicrobial resistance monitoring and biosafety on *P. mirabilis* and other pathogens found in poultry products

There are many studies on antimicrobial sensitivity on *Escherichia coli* in Algerian poultry samples but, on the other commensal Enterobacteria, no more data were founded. We hope that the findings of our study will contribute to serve as baseline information for the future ones.

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