Barka et al. *Afr. J. Clin. Exper. Microbiol.* 2021; 22 (2): 196 - 203 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Apr 2021; Vol.22 No.2
AJCEM/2075. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Antimicrobial resistance patterns and transferable traits in Enterobacteriaceae isolates from poultry in Tlemcen, Algeria

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Abstract:

Background: Antibiotics are overused in poultry industry, and this has resulted in the emergence of multidrug resistant (MDR) bacteria. The current study is aimed at determining antimicrobial resistance (AMR) patterns of Enterobacteriaceae isolates from poultry in the west of Algeria.
Methodology: Different chicken samples (kidney, bone and intestine) were collected and processed for culture using standard microbiological methods to isolate Enterobacteriaceae. Isolates were identified biochemically using API 20E, while isolated *Escherichia coli* was typed for O1, O2 and O78 antigens using slide agglutination with specific antisera. All identified isolates were tested against 26 antibiotic disks using the Kirby Bauer disk diffusion method according to the CLSI standards. The minimum inhibitory concentrations (MICs) of chloramphenicol, tetracycline, nalidixic acid, ofloxacin and ciprofloxacin were determined for selected isolates. Conjugative plasmid transfer, plasmid incompatibility and colicin tests were used to detect transferable resistance traits in 48 selected *E. coli* isolates.
Results: One hundred and thirty-eight bacteria species were isolated, which included *Escherichia* coli (n=107), *Salmonella* spp (n=11), *Klebsiella* spp (n=8), *Enterobacter* spp (n=7), *Pseudomonas* spp (n=3) and *Citrobacter* spp (n=2). Serotyping identified 24 agglutinable *E. coli* isolates with O78:K80 (n=11), O1:K1 (n=9) and O2:K1 (n=4). Antibiotic susceptibility showed high frequency of *E. coli* resistance to nalidixic acid (89.7%), tetracycline (82.2%), streptomycin (82.2%), nitrofurantoin (68.2%), ampicillin (45.8%), ticarcillin (44.9%), piperacillin (42.1%), and chloramphenicol (15.9%). The percentage of multi-drug resistance isolates (resistance to more than 3 antibiotic classes) was 87.9%. The results of conjugative transfer in 48 *E. coli* isolates shows that the most important resistance traits transferred by plasmids are *ASTeSuTmp* (18.5%) and *SuTmp* (12.3%).
Conclusion: This study confirmed the presence of multiple antibiotic resistant *E. coli* and other members of family *Enterobacteriaceae* in poultry in Algeria, and showed that these antibiotic resistance traits are easily disseminated by plasmids, with dire consequences on human health.

Keywords : Poultry, Enterobacteriaceae, antimicrobial resistance, conjugation, plasmid.

Received Aug 31, 2020; Revised Dec 31, 2020; Accepted Jan 1, 2021

Profils de résistance aux antimicrobiens et caractères transférables des isolats d'entérobactéries provenant de volailles à Tlemcen, Algérie

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Abstrait:

Contexte: Les antibiotiques sont surutilisés dans l'industrie de la volaille, ce qui a entraîné l'émergence de bactéries multirésistantes (MDR). L'étude actuelle vise à déterminer les profils de résistance aux antimicrobiens (RAM) des isolats d'Enterobacteriaceae provenant de volailles dans l'ouest de l'Algérie.

Méthodologie: Différents échantillons de poulet (rein, os et intestin) ont été prélevés et traités pour la culture en utilisant des méthodes microbiologiques standard pour isoler les Enterobacteriaceae. Les isolats ont été identifiés biochimiquement en utilisant l'API 20E, tandis que *Escherichia coli* isolé a été typé pour les antigènes O1, O2 et O78 en utilisant l'agglutination sur lame avec des antisérums spécifiques. Tous les isolats identifiés ont été testés contre 26 disques antibiotiques en utilisant la méthode de diffusion sur disque de Kirby Bauer selon les normes CLSI. Les concentrations minimales inhibitrices (CMI) du chloramphénicol, de la tétracycline, de l'acide nalidixique, de l'ofloxacine et de la ciprofloxacine ont été déterminées pour certains isolats. Des tests de transfert plasmidique conjugatif, d'incompatibilité plasmidique et de colicine ont été utilisés pour détecter des traits de résistance transférables dans 48 isolats sélectionnés d'*E. coli*.
Résultats: Cent trente-huit espèces de bactéries ont été isolées, parmi lesquelles *Escherichia coli* (n=107), *Salmonella* spp (n=11), *Klebsiella* spp (n=8), *Enterobacter* spp (n=7), *Pseudomonas* spp (n=3) et *Citrobacter* spp (n=2). Le sérotypage a identifié 24 isolats d'*E. coli* agglutinables avec O78: K80 (n=11), O1: K1 (n=9) et O2: K1 (n=4). La sensibilité aux antibiotiques a montré une fréquence élevée de résistance d'*E. coli* à l'acide nalidixique (89,7%), à la tétracycline (82,2%), à la streptomycine (82,2%), à la nitrofurantoïne (68,2%), à l'ampicilline (45,8%), à la ticarcilline (44,9%), à la pipéracilline (42,1%) et le chloramphénicol (15,9%). Le pourcentage d'isolats de résistance multi-médicaments (résistance à plus de 3 classes d'antibiotiques) était de 87,9%. Les résultats du transfert conjugatif dans 48 isolats d'*E. coli* montrent que les traits de résistance les plus importants transférés par les plasmides sont *ASTeSuTmp* (18,5%) et *SuTmp* (12,3%).
Conclusion: Cette étude a confirmé la présence de multiples *E. coli* résistants aux antibiotiques et d'autres membres de la famille des Enterobacteriaceae chez les volailles en Algérie et a montré que ces traits de résistance aux antibiotiques sont facilement disséminés par les plasmides, avec des conséquences désastreuses sur la santé humaine.

Mots clés: volaille, entérobactéries, résistance aux antimicrobiens, conjugaison, plasmide.

Introduction:

With a lower price than red meat, poultry is the most widespread meat consumed in Algeria. Poultry meat, like other meat can provide a good environment for microbial growth. Most members of the family Enterobacteriaceae have been known to be major cause of food-borne diseases and spoilage of a variety of foods, including poultry products (1,2). A large diversity of antibiotics is used in veterinary medicine to raise poultry in many countries (3,4), mostly through the oral route of antibiotic administration as prophylaxis or for the treatment of infectious diseases or in animal nutrition to promote growth and productivity (5,6). The excessive and misuse of such antimicrobials had led to increase in antibiotic resistance (7), which is considered critical and of high importance for human medicine (8,9,10).

Antimicrobial resistant pathogens in poultry infections may result in treatment failure, leading to economic losses, but also can be a source of resistant bacteria/genes that present a significant risk to human health (11). In the last decades, epidemics have been associated with resistant strains of food-borne Enterobacteriaceae (12). Avian Enterobacteriaceae are considered as secondary pathogens and mostly involved *Escherichia coli*. However, recently in Algeria, they are considered as one of the most important causes of economic losses in the poultry sector (13).

According to some reports, *E. coli* commonly found in raw meats, has the potential to transfer antibiotic resistance to other intestinal organisms and may act as transport medium for antimicrobial resistant genes to other pathogens (14,15,16,17). Antimicrobial resistant *E. coli* strains pose a serious problem for public health, since these strains could be passed to humans via the food chain or by direct contact with infected chicken (18). Therefore, the objectives of this study are to determine antimicrobial resistance patterns of Enterobacteriaceae isolates from poultry in Tlemcen, Algeria, and to detect the plasmids responsible for potential dissemination of resistant traits present in these isolates.

Materials and method:

Samples and isolation of bacteria species

Different chicken samples (kidneys, bones and intestines) were collected between 2018 and 2019 from various locations in western Algeria including Tlemcen, Oran, Sidi bellabes, Saida, Ain Temouchent and Naâma. From each sample, 1g was mixed with 9ml of Rappaport Vassiliadis broth (BioMérieux, Marcy l'Étoile, France), vortexed, and incubated overnight at 37°C. To isolate *E. coli* and *Salmonella* spp, a drop of the broth was streaked on Hektoen agar medium (Biokar, Diagnostics, Beauvais, France). Bromocresol purple lactose agar (Bio-Rad Laboratories Inc., California, USA) was used to isolate the other Enterobacteriaceae

Biochemical identification and serotyping

The isolates were identified biochemically using the API 20E system (BioMérieux, Marcy l'Étoile, France). All confirmed *E. coli* isolates were serotyped by the slide agglutination with specific antisera (Biovac, Angers,

France) for O1, O2, and O78 antigens in accordance with Qrskov and Orskov (19). The isolates confirmed as *Salmonella* were also serotyped (20) using an array of pooled and factor *Salmonella* antisera (Bio-Rad Laboratories Inc., California, USA).

Antibiotic susceptibility test (AST) assay

All identified isolates were tested for susceptibility to 26 antibiotics using the disk diffusion Kirby–Bauer standard method, with the following antibiotics; ampicillin (10µg), amoxicillin-clavulanic acid (10µg), ticarcillin (75µg), piperacillin (100µg), cefazoline (30 µg), cefoxitin (30µg), cefotaxime (30µg), cefepime (30µg), cefpirome (30µg), moxalactam (30µg), imipinem (10µg), gentamicin (10µg), amikacin (30µg), netilmycin (30µg), streptomycin (10µg), kanamycin (10µg), nitrofurantoin (300µg), nalidixic acid (30µg), ofloxacin (5µg), ciprofloxacin (5µg), colistin (10µg), tetracycline (30µg). chloramphenicol (30 µg), sulfonamide (300µg), trimethoprim (5µg), and sulfamethoxazole-trimethoprim (1.25/23.75 µg).

Isolates were categorized as sensitive or resistant to each antibiotic according to the Clinical and Laboratories Standards Institute guidelines (21). *E. coli* strain ATCC 25922 and *Pseudomonas aeruginosa* strain ATCC 27853 were used for quality control.

Minimum inhibitory concentrations

The minimum inhibitory concentrations (MICs) of chloramphenicol (Roussel, UCLAf), tetracycline (Sigma), nalidixic acid (Serva), ofloxacin (Roussel, UCLAf) and ciprofloxacin (Bayer) were determined for *E. coli* isolates (n=83) by the broth dilution technique according to Andrews (22).

Conjugative transfer experiment

A colony of selected donor isolates (isolates resistant to chloramphenicol and some multi-drug resistant *E. coli*) and a colony of the reference *E. coli* C600 Rif (host recipient strain) were put in each Brain Heart Infusion Broth (BHIB) tube and incubated for 4 hours at 35°C with stirring. 1 ml of donor and 1 ml of recipient cultures were mixed with a spreader in a Petri dish of Mueller Hinton broth and incubated overnight. 1 ml of sterile BHIB was added to the incubated mixtures and mixed with a spreader, and the supernatant containing the transconjugants was collected. A loopful of the supernatant was inoculated as a line on a quarter of the selective agar (which contain 2 antibiotics, one corresponding to the suspected resistant plasmid trait of the donor and the other to the chromosomal trait of the recipient) in Petri dish, and the mixture streaked along the remaining three quarters of the agar. After incubation for 18 hours at 35°C, an antibiogram was carried out on the transconjugants on non-selective agar in order to determine the characters transferred.

Test for plasmid incompatibility

In the test for incompatibility, the transconjugant was crossed with an *E. coli* which carries a reference plasmid, in order to determine the group to which the studied plasmid belongs. All the reference plasmids were from the "Institut Pasteur d'Algérie" (IPA) and includes; Com1 lfi 14R 525 resistant to kanamycin, Com1 PPED I resistant to chloramphenicol and trimethoprim, Com1 PPED 2 resistant to kanamycin, gentamicin, tobramycin and netilmycin, and FI Fi' R 386 resistant to tetracycline.

Test for colicin

The test isolate and control strain (*Escherichia coli* F3, which produces colicin) were stirred in BHIB for 4 hours at 35°C. A drop of the cultures was placed on Trypticase Soy agar (TSA, Bio-Rad Laboratories, Inc., California, USA), and incubated for 48 hours at 35°C. *Escherichia coli* J 5 Azide was grown in BHIB to obtain a slight cloudiness. A loopful of the growth was then diluted in 10 ml of physiological water. A drop of this dilution was added next to the test isolate and control strain on TSA, and agar incubated for 48 hours at 35°C. The production of colicin results by the isolate (and control strain) results in the formation of a zone of inhibition around *Escherichia coli* J 5 Azide, next to the isolate being studied and the positive control strain.

Statistical analysis

Statistical analysis of data and graphical representations were performed using XLSTAT Statistical Software version 2020.5.1 (www.xlstat.com).

Results:

Bacterial isolates

A total of 138 bacteria species were isolated; *E. coli* (n=107), *Salmonella* spp [n=11 with *S.* Gallinarum (n=7), *S.* Enteritidis (n=2), *S.* Infantis (n=1) and *S.* Brunei (n=1)], *Klebsiella* spp [n=8 with *K. oxytoca* (n=7) and *K. pneumoniae* (n=1)], *Enterobacter* spp [(n=7) with *E. cloacae* (n=4), *E. asburiae* (n=2) and *E. auraginus* n=1)], *Pseudomonas aeruginosa* (n=3) and *Citrobacter amalonaticus* (n=2). Serotyping of the *E. coli* identified 24 agglutinable isolates;

O78:K80 (n=11), O1:K1 (n=9) and O2:K1 (n=4).

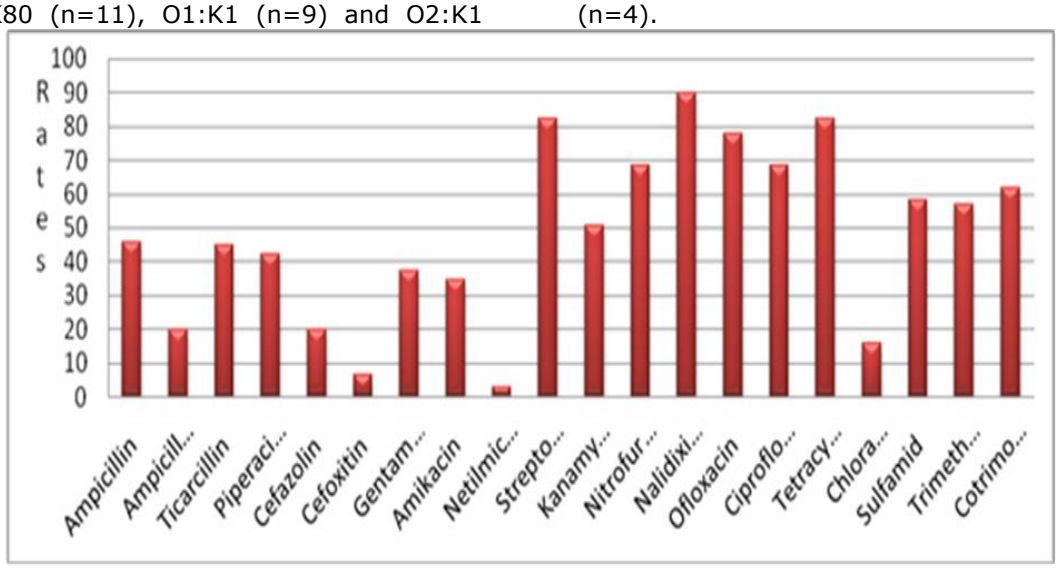

Figure-1 : Resistant patterns of *E.coli* isolated from poultry, n=107.

Results of antibiotic susceptibility test

The results of the disk diffusion AST on 107 *E. coli* isolates are shown in Fig 1. Resistance to aminoglycosides varied from 2.8% for netilmicin to 82.2% for streptomycin. Most of the *E. coli* isolates were resistant to tetracycline (82.2%) and nitrofurantoin (68.2%). There was also high resistance rate to ampicillin (45.8%), ticarcillin (44.9%), and piperacillin (42.1%). A worrying 15.9% resistance rate to chloramphenicol was obtained inspite of the fact this antibiotic is no longer used in veterinary medicine. Resistance rate to sulfonamides was 57.9%, fluoroquinolones 78.5%, and nalidixic acid 89.7%. All *E. coli* isolates were sensitive to cefepime, cefpirome, moxalactam, imipinem, ceftazidime and colistin. Multi-drug resistant isolates (resistance to more than 3 antibiotic classes) represented 87.9%

Salmonella isolates were resistant to nalidixic acid (63.6%), ciprofloxacin (63.6 %), ofloxacin (63.6%), nitrofurantoin (63.6 %), and streptomycin (27.3%). All *Enterobacter* isolates were resistant to ampicillin, amoxicillin-clavulanic acid, cefoxitin, cefazolin and nitrofurantoin, however no resistance to gentamicin, amikacin and kanamycin was observed, while 42.9% were resistant to nalidixic acid and ciprofloxacin. Regarding *Klebsiella* isolates, there was no resistance to gentamicin, amikacin and kanamycin, however all the isolates were resistant to ampicillin and tetracycline, 62.5% to streptomycin, 87.5% to nalidixic acid and nitrofurantoin, 75% to ciprofloxacin and 50% to ofloxacin. The minimum inhibitory concentrations of ciprofloxacin and tetracycline were respectively, 0.063 and 8µg/ml. At these MICs, 95.2% of tested isolates were resistant to ciprofloxacin and 95.3% to tetracycline. For nalidixic acid MIC of 8µg/ml, 40.6% of isolates were resistant and for ofloxacin MIC of 0.258 µg/ml, 15.6% isolates were resistant. For chloramphenicol MIC of 8µg/ml, 86.4% of isolates were resistant.

Transfer of resistant trait by conjugation

From the conjugation experiment on isolates resistant to chloramphenicol and multi-drug resistant *E. coli*, a total of 48 isolates transferred one or more markers. The results of the transfer showed that the most frequently transferred markers were *ASTeSuTmp* (18.5%) and *SuTmp* (12.3%) (Fig 2). However, *Tmp* was detected in 86.2%, *Te* in 50.8%, *Su* in 78.5% and *A* in 43.1% of the transconjugants.

The grouping of the plasmids allowed us to determine the *Inc* group to which all the plasmids belonged. With the exception of four plasmids (Figs 3, 4, 5, 6), all the plasmids were grouped into Com1 and F1 family. The colicin test revealed that of the 48 wild type isolates, 17 (35.4%) produced colicins while only 3 (6.3%) transconjugants were colicin positive.

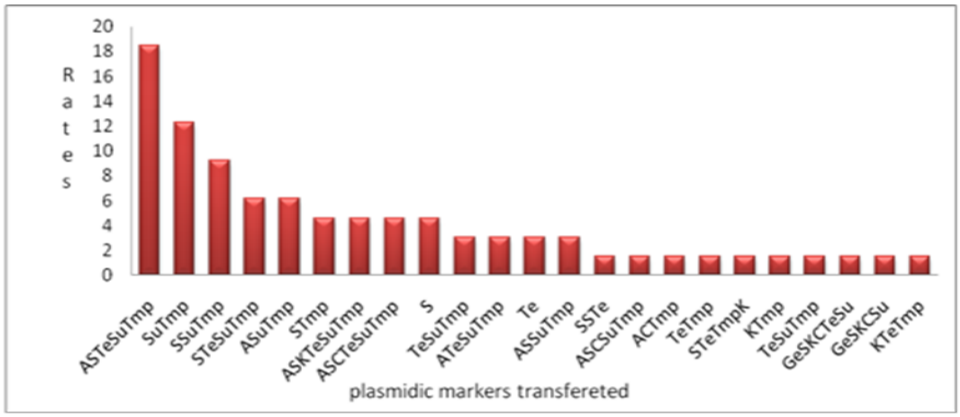

Figure-2 : Rates of plasmidic markers transfereted

A =Ampicillin, S=Streptomycin, Te=Tetracyclin, Su=Sulfamid, Tmp=Trimethoprim, K=Kanamycin, C=Chloramphenicol, Ge=Gentamycin

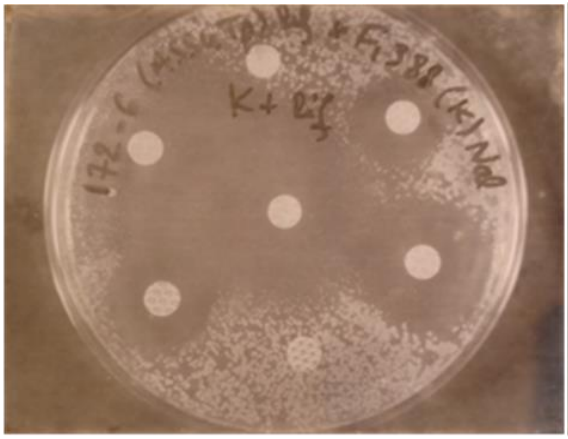

Figure-3: Grouping transconjugant *E.coli* 172-6 (ASSuTmp) in *E.coli* C600Rif with F1 386 (K) in *E.coli* K12 Nal, Selection dish K+Rif

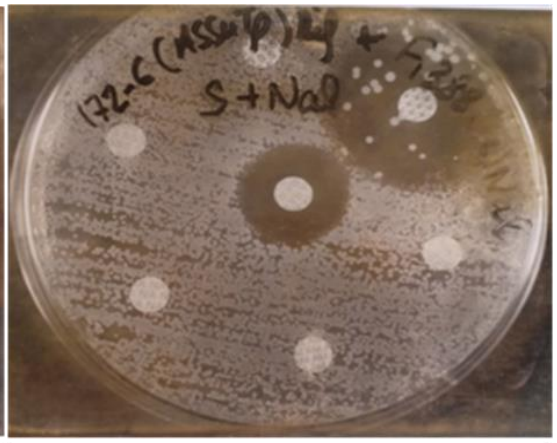

Figure-4: Grouping transconjugant *E.coli* 172-6 (ASSuTmp) in *E.coli* C600Rif with F1 386 (K) in *E.coli* K12 Nal, Selection dish S+Rif

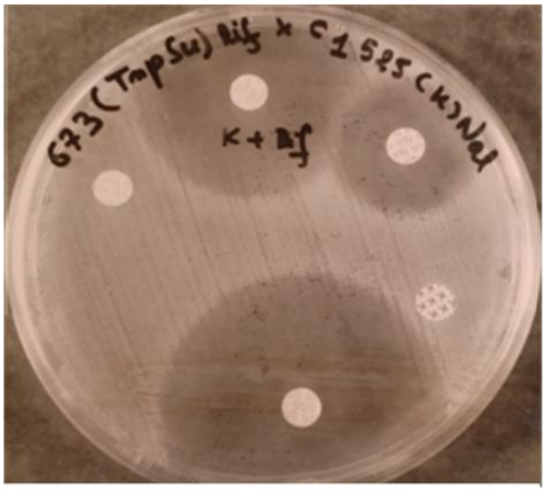

Figure-5: Grouping transconjugant *E.coli* 673 (SuTmp) in *E.coli* C600Rif with Com1 525 (K) in *E.coli* K12 Nal.

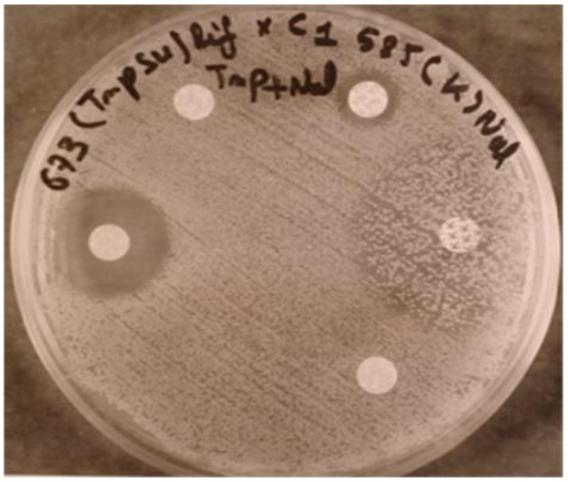

Figure-6: Grouping transconjugant *E.coli* 673 (SuTmp) in *E.coli* C600Rif with Com1 525 (K) in *E.coli* K12 Nal, in the opposite direction.

Discussion:

In this study, a total of 138 Enterobacteriaceae were isolated from different organs of poultry, with predominance of *E. coli*, and others such as *Salmonella*, *Klebsiella*, *Enterobacter*, *Pseudomonas* and *Citrobacter* in that order, similar to the results of the study by Boutaiba et al., (23). Of the 24 *E. coli* agglutinable isolates in our study, serotypes O78, O1 and O2 were identified at frequencies of 45.8%, 37.5% and 16.7% respectively, similar to the results observed in Algeria and Egypt (13,24). However, Ibrahim et al., (18) reported a lower prevalence of O78 (23.8%), O1 (14.9%) and O2 (12.6%) in their study. In Northern Ireland, *E. coli* serotype O78 was the predominant serotypes reported in chicken colibacillosis (25).

Most of the *E. coli* isolates exhibited multi-drug resistance phenotypes. The highest resistance rate was to nalidixic acid (89.7%) which is similar to the rate reported by Benameur et al., (26). Resistance to tetracycline, which is used as growth promoter or treatment of infections in domestic animals (27), is high at 82.2%. There was also high resistance of *E. coli* isolates to streptomycin (82.2%), ofloxacin (77.8%), ciprofloxacin (68.2%), nitrofurantoin (68.2%), sulfamethoxazole-trimethoprim (61.7%) ampicillin (45. 8%), ticarcillin (44.9%) and piperacillin (42.1%), which is similar to the studies by Bakhshi et al., (28) and Kim et al., (29) who reported that more that 60% of their isolates were resistant to tetracycline, streptomycin and ampicillin.

However, no resistance was detected for cefotaxime, cefepime, cefpirome, moxalactam, imipenem, ceftazidime and colistin, which is not unexpected given the fact that these classes of cephalosporins (and colistin) are not used in poultry industry. A 2012 study by Obeng et al., (33) in Australia reported a relatively lower resistance rates to tetracycline (40.6%), ampicillin (26.7%), and sulfamethoxazole-trimethoprim (12.4%), with no resistance (0%) to ceftiofur, ciprofloxacin and gentamicin, which is an indication of appropriate usage of these antibiotics in Australia. These findings however contradicted the report of a study in Zambia which showed 100% resistance to cefotaxime and ceftazidime (30).

The resistance rate of 37.4% to gentamicin in our *E. coli* isolates is also lower than the rates of 57.2% reported by Sciberras et al., (31) and 75.6% reported by Ahmed et al., (32). However, resistance rate of 15.9% to chloramphenicol in our isolates is rather too high for an antibiotic which use has been forbidden in animals, although higher resistance rates to chloramphenicol were recently reported in studies from Algeria (13, 23). The rate of multi-drug resistance in our *E. coli* isolates (resistance to more than 3 antibiotic classes) was extremely high at 87.9% but similar results were reported by Boutaiba et al., (23), where resistance rate of *E. coli* in the region of Tlemcen was higher compared to the other regions except for β-lactams where the region of Saida had the highest rate.

The resistance rate of *Salmonella* spp was 63.6% each to nalidixic acid, ciprofloxacin, ofloxacin and nitrofurantoin (furanes), and 27.3% to streptomycin. This may indicate an overuse of fluoroquinolones and furans antibiotics in the empirical treatment of suspected cases of salmonellosis on the part of breeders (34,35). All the *Enterobacter* isolates were resistant to ampicillin, amoxicillin-clavulanic acid, cefoxitin, cefazolin and furanes. On the other hand, no resistance was observed for gentamicin, amikacin and kanamycin, while 42.9% of the isolates were resistant to nalidixic acid and ciprofloxacin, which is similar to the findings of Halfaoui et al., (13). For *Klebsiella* isolates, no resistance was reported for gentamicin, amikacin and kanamycin but all the strains were resistant to ampicillin and tetracycline, 87.5% to furanes and nalidixic acid, 62.5% to streptomycin, 75% to ciprofloxacin, and 50% to ofloxacin, which are similar findings to the study by Burtram et al., (36).

With regards to the minimum inhibitory concentration of chloramphenicol, ciprofloxacin, tetracycline, nalidixic acid and ofloxacin, the resistance breakpoints were 8µg/ml, 0.0063µg/ml, 8µg/ml, 8µg/ml, and 0.25 µg/ml respectively. By this, the isolates were sensitive to the antibiotics tested except for tetracycline where intermediate resistance was found. The most frequently transferred resistance markers were *ASTeSuTmp* (18.5 %) and *SuTmp* (12.3%). However, the *Tmp* trait was present in 86.2%, *Te* (tetracycline) in 50.8%, *Su* (sulfamid) in 78.5% and *A* (ampicillin) in 43.1% of the transconjugants, similar to what Poirel et al., (38) reported. The high presence of these traits in the transconjugants can be explained by the fact that they are carried by easily disseminated characters, and the misuse of antibiotics as growth promoters and prophylaxis in animal husbandry, implies that there is always a reservoir of resistance and dissemination of the plasmids. However, traits such as nalidixic acid, ciprofloxacin and furans appeared not easily transferable as previously reported (38,39).

The grouping of the plasmids allowed us to determine the *Inc* group to which the plasmids belong, which are groups of the Com1 and F1 family, already described by Chaslus-Dancla et al., (40), with exception of 4 plasmids that we could not group. The colicin test was carried out on all the isolates which transferred antibiotic resistance trait. With the knowledge that the gene encoding colicin can be attached to antibiotic resistance gene (R plasmid), we investigated the production of colicin in the transconjugants obtained from the transfer of antibiotic resistance, and their wild type isolates. Of the 48 wild type isolates, 35.4% (n=17) produced colicins but only 3 (6.3%) transconjugants were colicins positive. These results led us to suppose that the antimicrobial resistance and production of colicin, are two different character traits carried by the same plasmid, even if it occurs at low frequency (41).

Conclusion:

This study confirmed the presence of multiple antibiotic resistant *E. coli* and other members of the family Enterobacteriaceae in poultry in Algeria, and showed that these transferable antibiotic resistance traits are easily disseminated by plasmids.

References:

1. Kamboh, A. A., Shoaib, M., Abro, S. H., Khan, M. A., Malhi, K. K., and Yu, S. Antimicrobial Resistance in Enterobacteriaceae Isolated from Liver of Commercial Broilers and Backyard Chickens. J Appl Poultry Res. 2018; 27 (4): 627–634. http://dx.doi.org/10.3382/japr/pfy045.
2. Gwida, M., Hotzel, H., Geue, L., and Tomaso, H. Occurrence of *Enterobacteriaceae* in raw meat and in human samples from Egyptian retail sellers. Int Sch Res Notices. 2014: 1-6. http://dx.doi.org/10.1155/2014/565671.
3. Landoni, M. F., and Albarellos, G. The use of antimicrobial agents in broiler chickens. Vet J. 2015; 205 (1): 21-27. doi:10.1016/j.tvjl.2015.04.016
4. Agunos, A., Leger, D., and Carson, C. Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada. Can Vet J. 2012; 53 (12): 1289-1300.
5. Page, S. W., and Gautier, P. Use of antimicrobial agents in livestock. Rev Sci Tech. 2012; 31 (1): 145-188. doi:10.20506/rst.31.1.2106.
6. Snary, E. L., Kelly, L. A., Davison, H. C., Teale, C. J., and Wooldridge, M. Antimicrobial resistance: a microbial risk assessment perspective. J Antimicrob Chemother. 2004; 53 (6): 906-917. doi.org/10.1093/jac/dkh182.
7. Mehdi, Y., Letourneau-Montminy, M. P., Gaucher, M. L., et al. Use of antibiotics in broiler production: Global impacts and alternatives. Anim Nutr. 2018; 4 (2): 170-178. doi: 10.1016/j.aninu.2018.03.002.
8. Gonzalez Ronquillo, M., and Angeles Hernandez, J. C. Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control. 2017; 72: 255-267.
9. World Health Organization. Critically Important Antimicrobials for Human Medicine. WHO, 5th Revision, 2017. http://www.who.int/foodsafety/publications/antimicrobials-fifth/en/
10. Diarra, M. S., Rempel, H., Champagne, J., Masson, L., Pritchard, J., and Topp, E. Distribution of antimicrobial resistance and virulence genes in *Enterococcus* spp and characterization of isolates from broiler chickens. Appl Environ Microbiol. 2010; 76 (24): 8033-8043.
11. Nhung, N. T., Chansiripornchai, N., and Carrique-Mas, J. J. Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review. Front Vet Sci. 2017; 10 (4): 126. doi:10.3389/fvets.2017.00126.
12. Mather, A. E., Reid, S. W. J., Maskell, D. J., Parkhill, J., Fookes, M. C., and Harris, S. R. Distinguishable epidemics of multidrug-resistant *Salmonella* Typhimurium DT104 in different hosts. Science. 2013; 341 (6153):1514-1517. doi:10.1126/science.1240578.
13. Halfaoui, Z., Menoueri, N. M., and Bendali, L. M. Serogrouping and antibiotic resistance of *Escherichia coli* isolated from broiler chicken with colibacillosis in center of Algeria. Vet World. 2017; 10 (7): 830-835.
14. Kilonzo-Nthenge, A., Rotich, E., and Nahashon, S. N. Evaluation of drug-resistant Enterobacteriaceae in retail poultry and beef. Poult Sci. 2013; 92 (4): 1098–1107. doi: 10.3382/ps.2012-02581.
15. Akond, M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Am J Environ Sci. 2009: 5 (1): 47–52.doi: 10.3844/ajessp.2009.47.52.
16. Dunowska, M., Morley, P. S., Traub-Dargatz, J. L., Hyatt, D. R., and Dargatz, D. A. Impact of hospitalization and antimicrobial drug administration on antimicrobial susceptibility patterns of commensal *Escherichia coli* isolated from the faeces of horses. J Am Vet Med Assoc. 2006; 228 (12): 1909–1917. doi: 10.2460/javma.228.12.1909.
17. Schroeder, C. M., White, D. G., Ge, B., et al. Isolation of antimicrobial resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. Int J Food Microbiol. 2003; 85 (1-2):197-202.
18. Ibrahim, R. A., Cryer, T. L., Lafi, S. Q., AbuBasha, E., Good, L., and Tarazi, Y. H. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. Vet Res. 2019; 15 (1): 159. https://doi.org/10.1186/s12917-019-1901-1.
19. Qrskov, F., and Orskov, I. Serotyping of *Escherichia coli*. Method Microbiol. 1984; 14: 43-112.
20. Grimont, P. A. D., and Weill, F. X. Antigenic formulae of the *Salmonella* serovars. 9th ed. France, Institut Pasteur, 2007.
21. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-six Informational Supplement Document. M100-S26. 26th ed. CLSI, Wayne, PA. 2016.
22. Andrews, J. M. Determination of Minimum inhibitory concentrations. J Antimicrob Chemother. 2001; 48 (Suppl S1): 5-16.
23. Boutaiba Benklaouz, M., Aggad, H., and Benameur, Q. Resistance to multiple first-line antibiotics among *Escherichia coli* from poultry in Western Algeria. Vet World. 2020; 13 (2): 290-

295. doi:10.14202/vetworld.2020.290-295
24. Seifi, S., Khoshbakht, R., and Atabak, A. R. Antibiotic susceptibility, serotyping and pathogenicity evaluation of avian *Escherichia coli* isolated from broilers in northern Iran. Bulg J Vet Med. 2015; 18 (1): 74–82. doi.org/ 10.15547/bjvm.8.
25. Mcpeake, S. J. W., Smyth, J. A., and Ball, H. J. Characterisation of avian pathogenic *Escherichia coli* (APEC) associated with colisepticaemia compared to faecal isolates from healthy birds. Vet Microbiol. 2005; 110 (3–4): 245-253. doi.org/10.1016/j.vetmic.2005.08.001.
26. Benameur, Q., Guemourb, D., Hammoudi, A., et al. Antimicrobial resistance of *Escherichia coli* isolated from chickens in West of Algeria. Int J Sci Basic Appl Res. 2014; 13 (1): 366-370.
27. Chopra, I. New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors. Drug Resist Update. 2002; 5 (3-4): 119-125. doi:10.1016/s1368-7646(02)00051-1.
28. Bakhshi, M., Fatahi Bafghi, M., Astani, A., Ranjbar, V.R., Zandi, H., and Vakili, M. Antimicrobial resistance pattern of *Escherichia coli* isolated from chickens with colibacillosis in Yazd. J Food Qual Hazards Control. 2017; 4 (3): 74-78.
29. Kim, T. E., Jeong, Y. W., Cho, S. H., Kim, S. J., and Kwon, H. J. Chronological study of antibiotic resistance and their relevant genes in Korean avian pathogenic *Escherichia coli* isolates. J Clin Microbiol. 2007; 45 (10): 3309-3315.
30. Chishimba, K., Hang'ombe, B. M., Muzandu, K., et al. Detection of extended-spectrum beta-lactamase-producing *Escherichia coli* in market-ready chickens in Zambia. Int J Microbiol. 2016; 1-5. doi:10.1155/2016/5275724.
31. Sciberras, M., Pipova, M., Regecova, I., Jevinova, P., and Demjanova, S. Antibiotic Resistance of *Escherichia Coli* Isolated from Broiler Chickens. Folia Vet. 2019; 63 (3): 1-8. doi:10.2478/fv-2019-0021.
32. Ahmed, A. M., Shimamoto, T., and Shimamoto, T. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int J Med Microbiol. 2013; 303 (8): 475 - 483 doi:10.1016/j.ijmm.2013.06.009.
33. Obeng, A. S., Rickard, H., Ndi, O., Sexton, M., and Barton, M. Antibiotic resistance, phylogenetic grouping and virulence potential of *Escherichia coli* isolated from the faeces of intensively farmed and free-range poultry. Vet Microbiol. 2012; 154 (3-4): 305 - 315. doi:10.1016/j.vetmic.2011.07.010.
34. Fallah, S. H., Asgharpour, F., Naderian, Z., and Moulana, Z. Isolation and Determination of Antibiotic Resistance Patterns in Non-typhoid *Salmonella* spp isolated from chicken. Int J Enteric Pathog. 2013; 1 (1): 17-21 doi:10.17795/ijep9416.
35. Bada-Alambedji, R., Fofana, A., Seydi, M., and Akakpo, A. J. Antimicrobial resistance of *Salmonella* isolated from poultry carcasses in Dakar (Senegal). Braz J Microbiol. 2006; 37: 510-515.
36. Burtram, C. F., Mnabisa, A., Gouws, P. A., and Morris, T. Antimicrobial-resistant *Klebsiella* species isolated from free-range chicken samples in an informal settlement. Arch Med Sci. 2012;8(1):39-42. doi:10.5114/aoms.2012.27278.
37. Chaslus-Dancla, E., Gerbaud, G., Lagorce, M., Lafont, J. P., and Courvalin, P. Persistence of an antibiotic resistance plasmid in intestinal *Escherichia coli* of chickens in the absence of selective pressure. Antimicrob Agents Chemother. 1987; 31 (5): 784-788.
38. Poirel, L., Madec, J., Lupo, A., et al. Antimicrobial Resistance in *Escherichia coli*. Microbiol Spectr. 2018; 6 (4): ARBA-0026-2017. doi:10.1128/microbiolspec.ARBA-0026-2017.
39. Wooley, R. E., Spears, K. R., Brown, J., Nolan, L. K., and Dekich, M. A. Characteristics of conjugative R-plasmids from pathogenic avian *Escherichia coli*. Avian Dis. 1992; 36 (2): 348-352.
40. Chaslus-Dancla, E., Lafont, J. P., and Guillot, J. F. *Inc* groups among plasmids harbored by *Escherichia coli* of avian origin. Ann Microbiol. 1980; 131B (2): 203-206.
41. Blanco, J. E., Blanco, M., Mora, A., and Blanco, J. Production of toxins (enterotoxins, verotoxins, and necrotoxins) and colicins by *Escherichia coli* strains isolated from septicemic and healthy chickens: relationship with in vivo pathogenicity. J Clin Microbiol. 1997; 35 (11): 2953-2957