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ESBL/Cephalosporinase-producing Escherichia coli from retail poultry meat in Tunisia: Predominance of *bla*$_{CTX-M}$ gene and multidrug resistance

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Abstract

In the present study, 80 samples of chicken meat collected from various super markets in Tunis City, Tunisia, were analyzed for detection of ESBL-, pAmpC-producing *E. coli*. Thirteen cefotaxime resistant *E. coli* isolates were recovered. The majority of these isolates were resistant to amoxicillin, amoxicillin/clavulanic acid, ceftazidime and cefotaxime and a positive synergy was detected in 23 isolates, however, resistance to imipenem was not observed. The majority of these isolates were multidrug-resistant (tetracycline, streptomycin, trimethoprim / sulfamethoxazole). According to PCR investigating of bla genes (*blaCTX-M*, *bla*TEM, *bla*SHV), *blaCTX-M* gene was detected in 27 isolates, 4 out them contained the *bla*TEM gene, and one co-harbored the *bla*SHV gene. One isolate contained only the *bla*TEM gene. Two isolates did not show any of the investigated genes. These finding suggest that raw chicken retail meats are highly contaminated with ESBL-producing *E. coli* implementing a great risk to human health in Tunisia.

Keywords: *Retail Chicken Meat; E. Coli; Esbl; Multi-Drug Resistance*

Introduction

Plasmid-mediated beta-lactamases (extended spectrum beta-lactamases (ESBLs) and AmpC β-lactamases (pAmpC)) have been of great concern worldwide. The rapid increase in the proportion of ESBL-producing *E. coli* in alimentary tract of food producing and pet animals have been occurred globally since 2000s [1]. Therefore, prevalence of ESBL-producing *E. coli* has been also appeared to be in increase in various foodstuffs including raw milk, dairy products and meat, particularly chicken meat [2]. Retail chicken meat contaminated by *E. coli* producing ESBLs or pAmpC has been increasingly reported worldwide [3,4]. Many reports have described the close genetic relatedness of *E. coli* isolates of animal and human origins. Such transmission (animal to human) is likely occurred by physic contacts or by consummation of food products containing resistant *E. coli* isolates. This phenomenon likely contributes to the increased incidence of infections with these bacteria in humans [2,5]. The aims of this study were investigate the occurrence of ESBL/cephalosporinase-producing *E. coli* in retail chicken meat in Tunisia and to investigate ESBL-encoding genes in the collected isolates.

Materials and Methods

Sample collection and bacterial isolation

A total of 80 poultry meat were purchased from three local markets in Tunis City, Tunisia, from January to April 2016. For bacterial isolation, 25 g of each sample was inoculated into 225 mL of buffered peptone water and

incubated at 37 °C for 18 h. One milliliter of each culture was then inoculated on Tryptone-Bile-Glucuronic Agar (TBX Agar) (HiMedia Laboratories) containing 2 mg/L cefotaxime and cultured at 37 °C for 24 h. From each plate, one colony with typical morphology of *E. coli* was selected and identified by Api20E (Bio-Mérieux, France).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of bacterial isolates was determined using the agar diffusion method according to the Clinical and Laboratory Standards Institute [6]. *E. coli* ATCC 25922 was used for quality control. Isolates were screened for the ESBL-producing phenotype by the standard double-disc synergy test, as described previously [7]. The following antimicrobial discs (Oxoid) were used: amoxicillin/clavulananic acid (30/10 µg), cefotaxime (30 µg), cefepime (30 µg), ceftazidime (30 µg) and aztrenam (30 µg). For quality control, *Klebsiella pneumoniae* ATCC 700603) was used.

Characterization of ESBL-encoding genes

Bacterial DNA was extracted by boiling 3 to 5 colonies in 500 µL of sterile water during 10 min. The extracted DNA was used as the template for PCR amplification of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes encoding the most common ESBL enzymes as previously reported [8,9]. Gel electrophoresis was used for the analysis of PCR products using 2 % agarose gel and ethidium bromide (0.5 µg/mL) staining.

Results and discussion

In Tunisia and worldwide, livestock have been recognized as important reservoir of antibiotic-resistant zoonotic bacteria such as methicillin-resistant *Staphylococcus aureus*, ESBL-producing *Enterobacteriaceae* (mainly *E. coli*) and vancomycin-resistant enterococci [10]. Resistant bacteria are transferred to human via food chain or direct contact with animals. In our work, 30 (37.5 %) out of the 80 samples of chicken meat contained cefotaxime-resistant *E. coli* isolates (Table 1). This prevalence for carriage of cefotaxime-resistant *E. coli* is compared to other ones reported worldwide, highlighting the importance of poultry and poultry meat as reservoir of extended-sprectrum cephalosporin-resistant *E. coli* [11,12]. A positive synergy test was observed in 23 isolates, and therefore was classified as ESBL producers. The remaining 7 isolates were resistant to amoxicillin, amoxicillin-clavulanic acid, ceftazidim, cefotaxime and cefoxitine. This phenotype is typical to cephalosporinase production; however, this do not means that these isolates are not ESBL producers. Indeed, co-production of ESBLs and plasmidic cephalosporinases is common in *E. coli* from human and animal origins [3,11]. Antibiotic susceptibility showed that the majority of isolates were multidrug resistant, being resistant not only to beta-lactams but also to nalidixic acid, ciprofloxacin, tetracycline, streptomycin, and trimethoprim / sulfamethoxazole. This multiresistance trait has been increasingly reported in *E. coli* from poultry and poultry meat worldwide as well as in Tunisia [13,14]. It seems that for infections caused by such multi-drug resistant *E. coli* isolates, therapeutic options are limited and might be restricted to aminosides (gentamicin, amilakacin) and to imipenem (all our isolates were susceptible to these antibiotics). According to PCR investigating of commonly reported *bla* genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV}), the bla_{CTX-M} gene was detected in 27 isolates, 4 out them contained the bla_{TEM} gene, and one co-harbored the bla_{SHV} gene. In addition one isolate contained only the bla_{TEM} gene; however, two isolates with negative synergy test harbored none of these genes (Table 1). Unfortunately, Owing to many financial limitations we were unable to sequence these genes in order to know the exact variants of these genes. However, according to the epidemiology of avian ESBL-producing *E. coli* isolates, we can speculate the occurrence of $bla_{CTX-M-1}$ in our isolates [13-15]. The epidemiology of genes encoding ESBLs has changed dramatically by the beginning of the 20st centry. Indeed, during the last 17 years ESBLs encoded by the classical TEM- and SHV-ESBLs have increasingly decreased to give way to *CTX-M*- enzymes. Currently, *CTX-M* enzymes are pandemic and were identified from patients, healthy humans, livestock, companion animals, food products and sewage [14]. Three isolates harbored the bla_{CTX-M} and one harbored the bla_{TEM} were cephalosporinase producers par the synergy test; it seems that these isolates harbored also a plasmidic AmpC cephalosporinase enzyme such as blaCMY-2 as reported previously [14]. In conclusion, our results showed high prevalence of ESBL/cephalosporinase-producing *E. coli* isolates from retail chicken meat. Isolates were multi-drug resistant, and the majority harbored a bla_{CTX-M} gene and some of them most likely co-harbored a plasmidic AmpC gene. The spread of such isolates in the environment and to Human is worrisome, since therapeutic options against them are limited.

Isolates	Antibiotic resistance profiles*	Synergy test	Genes
Ec.1	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.2	NAL, CIP, SXT, TET, S, AMX, AMC, FOX, CAZ, CTX	-	*CTX-M*
Ec.3	NAL, CIP, SXT, TET, AMX, AMC, FOX, CAZ, CTX	-	*CTX-M*
Ec.4	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.5	NAL, CIP, SXT, TET, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.6	NAL, CIP, SXT, TET, AMX, AMC, FOX, CAZ, CTX	-	-
Ec.7	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*+SHV
Ec.8	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.9	NAL, CIP, SXT, TET, S, CO, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.10	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX.	+	*CTX-M*
Ec.11	NAL, CIP, SXT, TET, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.12	NAL, CIP, SXT, TET, AMX, AMC, FOX, CAZ, CTX	-	*CTX-M*
Ec.13	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.14	NAL, CIP, SXT, TET, AMX, AMC, FOX, CAZ, CTX	-	*CTX-M*
Ec.15	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.16	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*
Ec.17	NAL, CIP, SXT, TET, AMX, AMC, FOX, CAZ, CTX	-	-
Ec.18	NAL, CIP, CO, AMX, AMC, CTX	+	*CTX-M*
Ec.19	NAL, CIP, SXT, TET, S, AMX, AMC, CAZ, CTX	+	*CTX-M*+TEM
Ec.20	NAL, CIP, SXT, TET, S, AMX, FOX, CAZ, CTX	+	*CTX-M*
Ec.21	NAL, CIP, SXT, TET, S, CO,AMX, AMC, FOX, CAZ, CTX	-	TEM
Ec.22	NAL, CIP, SXT, TET, S, CO, AMX, CAZ, CTX	+	*CTX-M*
Ec.23	NAL, CIP, SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M*
Ec.24	SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M* +TEM
Ec.25	SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M* +TEM
Ec.26	SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M* +TEM
Ec.27	SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M*
Ec.28	SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M*
Ec.29	SXT, TET, S, AMX, CAZ, CTX	+	*CTX-M*
Ec.30	SXT, TET, AMX, CAZ, CTX	+	*CTX-M*

* NAL: nalidixic acid, CIP: ciprofloxacin, SXT : trimethoprim / sulfamethoxazole, TET : tetracycline, S: streptomycin, CO : colistin, AMX: amoxicillin, AMC: amoxicillin-clavulanic acid, FOX : cefoxitin, CAZ : ceftazidime, CTX : cefotaxime

Table 1: Characteristics of cefotaxime-resistant *E. coli* isolates

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