Epidemiology of Colistin Resistant & ESBL producing Gram-negative bacilli in Lebanese Chicken and Swine Farms

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The rise of multidrug resistance in Gram-negative bacilli is nowadays considered a serious challenge encountered by healthcare professionals. Resistance in Gram-negative bacilli is mainly mediated via the production of extended spectrum beta-lactamases (ESBL), AmpC beta-lactamases and carbapenemases. Genes encoding these enzymes are often co-localised on plasmids harbouring resistance genes to other commonly prescribed antibiotics in human medicine such as aminoglycosides and quinolones[1]. Infections with these multidrug-resistant organisms (MDROs) could thus pose therapeutic challenges when encountered. This is currently emphasised with the recent emergence of colistin resistance in Gram-negative bacilli. Before 2015, colistin resistance was thought to be only mediated through chromosomal mutations that lead to the alteration of the lipid A subunit of the lipopolysaccharides chain[2]. However, in 2016 Liu *et al.* reported the first detection of a transferable phosphoenolamine transferase named *mcr-1* gene in *E. coli* strains isolated from pigs and meat. Thereafter, *mcr* variants became heavily reported in humans and animals across all continents.[3]

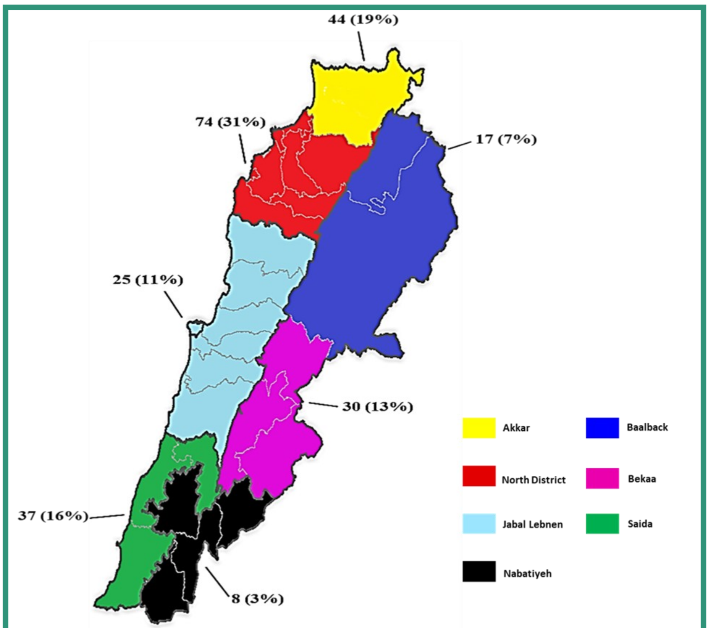

Figure. Prevalence of MDROs in Lebanese poultry farms. Prevalence is expressed as the "Number of isolates (%)"

Nowadays, farm animals are considered a reservoir of antimicrobial resistance. The major public health concern about MDROs spread in animals is their potential transmission to humans. Once transmitted, these organisms can cause infections with limited therapeutic options, especially those cross-resistant to antibiotics frequently used in human medicine. In Lebanon, the dissemination of MDROs in the clinical setting is well documented[4] however, studies addressing multidrug resistance in animals remain scarce. In collaboration with the Ministry of Agriculture, we undertook this study with the aim of determining the prevalence of extended-spectrum cephalosporin and colistin-resistant Gram-negative bacilli in Lebanese chicken and swine farms.

Between August and December 2015, 981 faecal swabs were obtained from 49 poultry farms distributed across Lebanon. In May 2017, 114 faecal samples were collected from swine farms located in south Lebanon. Separate media supplemented with cefotaxime, ertapenem, and colistin were used for the screening of resistant organisms. Double-disk synergy test, AmpC disk test and Carba NP test were performed to detect ESBL, AmpC and carbapenemase producers, respectively. Detection of beta-lactamase and *mcr* genes was performed using real-time polymerase chain reaction.

In 2015, out of 981 faecal swabs collected, 203 (21%) showed bacterial growth on the selective medium supplemented with cefotaxime (**Figure**)[5]. In total, 235 strains were isolated: 217 were identified as *E. coli* (92%), 8 as *K. pneumoniae* (3%), 3 as *P. mirabilis* (1%) and 3 as *E. cloacae* (1%). Multilocus sequence typing (MLST) analysis of *E. coli* isolates showed the presence of different sequence types distributed across the country: ST156, ST5470, ST354, ST155 and ST3224. The phenotypic tests revealed that 44%, 28% and 20% of the strains were AmpC, ESBL, AmpC/ESBL producers, respectively. The putative TEM gene was detected in 83% of the isolates, SHV in 20%, CTX-M in 53% and CMY AmpC b-lactamase gene in 65%. Moreover, during this surveillance study, an *mcr-1*-positive colistin-resistant *E. coli* strain was isolated from the south of Lebanon[6]. This *E. coli* isolate was an ESBL producer harbouring the TEM-135-like gene. MLST analysis revealed that this strain is of the sequence type ST515. This ST differs from those previously reported in *E. coli* isolates harbouring the *mcr -1* gene in food-producing animals[6]. As for the swine farms, out of 114 faecal samples, 76 showed growth on the medium with cefotaxime. In total, 111 strains were isolated with 94% being *E. coli*[7]. Phenotypic tests showed that 98, 6 and 7 strains were ESBL, AmpC, and ESBL/AmpC producers, respectively. CTX-M and CMY were the main beta-lactamase genes detected. In parallel, on the medium supplemented with colistin, 19 samples showed growth. From these, 23 colistin-resistant *E. coli* strains harbouring the *mcr-1* gene were isolated (**Table**). [4]

	Isolate	Colistin MIC (µg/ml)	AMP	FOX	ATM	CTX	TZP	FEP	AUG	CAZ	Carb	GNT	SXT	CIP	TGC	*bla* genes
Farm 1	*E.coli* (1)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (2)	4	R	S	S	S	S	S	R	S	S	S	R	R	S	
	E.coli (3)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (4)	16	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (5)	8	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (6)	8	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (7)	8	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (8)	4	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (9)	4	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (10)	4	R	R	S	S	S	S	R	S	S	R	R	S	S	SHV/TEM
Farm 2	*E.coli* (11)	8	R	S	S	S	S	S	R	S	S	S	R	S	S	
	E.coli (12)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (13)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (14)	8	R	S	S	S	S	S	S	S	S	S	S	S	S	
	E.coli (15)	8	R	R	S	S	S	S	R	S	S	S	S	S	S	SHV/TEM
Farm 3	*E.coli* (16)	8	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (17)	8	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (18)	4	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (19)	8	R	S	S	S	S	S	S	S	S	S	R	S	S	
	E.coli (20)	8	R	S	S	S	S	S	S	S	S	S	R	S	S	
	E.coli (21)	16	R	R	S	S	S	S	R	R	S	S	R	S	S	CTX-M/SHV/TEM
	E.coli (22)	8	R	S	S	S	S	S	S	S	S	S	S	R	S	
	E.coli (23)	up 256	R	S	S	R	S	R	S	R	S	S	S	R	S	CTX-M/SHV/TEM

Table. Resistance profiles of mcr-1 colistin-resistant *E. coli* strains isolated from the Lebanese swine farms

Our work illustrates the current epidemiology of multidrug resistant Gram negative bacilli in Lebanese chicken farms. ESBL and AmpC producers cross-resistant to antibiotics used in human medicine are highly prevalent across the territory. As demonstrated by Olaitan et al., *mcr-1*-harbouring strains can be readily spread from animals to the human gut and thus our finding sparks concerns over the transmission of *mcr-1* strains to the Lebanese community. Nowadays, carbapenem-resistant isolates are disseminated in the clinical and community settings in Lebanon. This dissemination has necessitated the frequent use of colistin in Lebanese hospitals. Therefore, it is expected that *mcr -1* strains, when transmitted from animals to humans in Lebanon, will be easily selected and further diffused by the selective pressure applied by the use of colistin and other antibiotics in clinical settings[7]. Surveillance studies addressing the current epidemiology of colistin resistance are thus warranted in Lebanon. In addition, the usage of colistin in veterinary medicine should be re-evaluated, as unpublished data have revealed its heavy use in animals in Lebanon.

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