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MOLECULAR CHARACTERIZATION OF RESISTANCE TO EXTENDED-SPECTRUM β-LACTAMS IN KLEBSIELLA PNEUMONIAE AND KLEBSIELLA OXYTOCA ISOLATES FROM MEAT AND MEAT PRODUCTS

By

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ABSTRACT

Prevalence of *K. pneumoniae* and *K. oxytoca* in meat and meat products was estimated in the present study. Four-hundred seventy samples of meat and meat products. (40 imported frozen minced meat,35 imported frozen meat, 25 local meat, 24 Local minced meat, 34 kofta, 46 sausage, 37 hot dog, 29 canned beef, 106 luncheon, 43 basterma, 51 beef burger) were collected randomly from different retail shops. The prevalence of *K. oxytoca* was higher in meat (9.7%) than in meat products (7.9%), while the rate of isolation of *K. pneumoniae* was the same in meat and meat products (11.3%). The isolation rate was higher in imported minced meat (10% for *K. oxytoca* and 15% for *K. pneumoniae*) in comparison with the local minced meat (*K. oxytoca* 8.3% and *K.pneumoniae*12.5%). All samples of canned beef were negative. The highest isolation rate among the meat product samples was from luncheon (11.3% *K. oxytoca* and 16.0% *K. pneumoniae*) and basterma (9.3% *K. oxytoca* and13.9% *K.pneumoniae*) and the lowest was in beef burger **(**3.9% *K. oxytoca* and7.8% *K. pneumoniae*)**.** Twelve *K. pneumoniae* and *K. oxytoca* isolates were investigated for antimicrobial resistance against β-lactams groups of antibiotics. The resistance of the isolates to cephalothin was 100%, ampicillin 91.7%, cefpodoxime 75%, cefotaxime 66.7%, sulfamethazole 41.7%, ceftazidime 33.3%, ceftriaxone16.7%, imipenem and cefepime 8.3%. The 12 isolates of *Klebsiellae* (5*K. oxytoca* and 7 *K. pneumoniae*) were tested for PEH gene (gene of identification of *K oxytoca*) and β-lactam resistance genes (*shv*, *tem*, *ctx-m*). The *shv* gene was detected in 12 (100%) of *K. pneumoniae* and *K. oxytoca* isolates, *tem* gene was detected in 11 (91.7%) isolates (5 isolates of *K. oxytoca* and 6 isolates of *K. pnemoniae*); while *ctx-m* gene was detected in 9(75%) isolates (4 isolates of *K. oxytoca* and 6 isolates of *K. pnemoniae*).

Key words:

Klebsiella pneumoniae, Klebsiella oxytoca, imported frozen meat, local meat, meat products, extended spectrum beta-lactamase, Antimicrobial resistances.

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INTRODUCTION

Klebsiella is well known to most clinicians as a cause of community-acquired bacterial pneumonia. As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals, who are hospitalized and suffer from severe underlying diseases **(Podschun and Ullmann, 1998).** Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus *Klebsiella*. *K. pneumoniae* causes a necrotizing process with a predilection for debilitated people **(Umeh and Berkowitz, 2002).** *Klebsiella pneumoniae* infections may occur at almost all body sites, but the highest incidence is found in the urinary and respiratory tract **(regue *et al.*, 2004).** *Klebsiella oxytoca* was reported as an enterotoxigenic microorganism and causes haemorrhagic colitis **(Gundogan *et al.*, 2011).** *Klebsiella* spp. is commonly found in the environment and the gastrointestinal tracts of animals **(Haryani *et al.*, 2007)**. Antibiotic resistance is a serious problem in clinical medicine. The efficacy of treatment with the widely used beta-lactam antibiotic is constantly challenged by the emergence of new resistant bacterial strains. In the recent years, a substantial increase in antibiotic resistance has been observed, mainly in developing countries, because of self-medication, the suboptimal quality of bacteria can be transferred to pathogenic species **(Lester *et al.*, 1990, Doucet *et al.*, 1992; 2001)**. Food animals are increasingly recognized as a reservoir for extended-spectrum-β -lactamases (ESBL) producing strains, which can be transmitted via the food chain. Faecal contamination might occur during animal slaughtering, milking, and/or processing, and the growth of the contaminating bacteria may occur during the product transport and storage phases. Consequently, without good hygienic practices, foods may act, as a vehicle of transfer of β-lactam-resistant bacteria to the gastrointestinal tract of consumers **(Overdevest *et al.*, 2011)** .β-lactamase production is the predominant mechanism for resistance to β-lactam antibiotics in Gram-negative bacteria. Extended-spectrum β-lactamases (ESBL) were first described in the 1980 and they have been detected in *Klebsiella* species, and later in other

Gram-negative bacteria **(Kiratisin *et al.*, 2008; Cheng *et al.*, 2008)**. ESBL are enzymes that are often plasmid mediated and confer broad resistance to penicillins, cephalosporins and monobactams. They are derivatives of plasmid-mediated *tem* and *shv* β-lactamases genes. Extended-spectrum-β-lactamases genes are undergoing continuous mutation, causing the development of new enzymes showing expanded substrate profiles. At present, there are more than 300 different ESBL genes, and these have been clustered into nine different structural and evolutionary families based on amino acid sequence. *tem* and sulphydryl variable *shv* were the major types. However, *ctx-m* type is more common in some countries **(Paterson *et al.*, 2003)**. Determination of *tem* and *shv* genes by molecular techniques in Extended-spectrum-β-lactamase producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology **(Jain and Mondal, 2008).** Therefore**,** the current study was conducted to evaluate the diversity of extended-spectrum β-lactamases genes in *Klebsiella oxytoca* and *Klebsiella pneumoniae* isolated from meat and meat products collected from different sources.

MATERIAL AND METHODS

Samples:

A total 470 meat and meat product samples (40 imported frozen minced meat, 35 imported frozen meat, 25 local meat, 24 local minced meat, 34 kofta, 46 sausage, 37 hot dog, 29 canned beef, 106 luncheon, 43 basterma, 51 beef burger) was randomly collected from groceries, super markets and butcher's shops in Cairo and Giza governorates during the period 2013-2014. Samples were collected under aseptic condition in sterile polyethylene bags and transferred directly to the laboratory in iceboxes as soon as possible.

Media and kits used for isolation and biochemical identification:

Nutrient broth, Eosin-methylene blue agar, MacConkey agar, Tryptone Soya broth, Tryptone Soya agar, Muller Hinton broth, Muller Hinton agar were used for isolation and API20E kits (bio Mérieux) were used for biochemical identification.

Oligonucleotide primers used for detection of beta-lactamase genes:

Three sets of Oligonucleotide primers sequences were used for detection of beta-lactamase genes, namely *shv*, *tem* and *ctx* genes (Table 1) and 2 genes, *peh*-C and *peh*-D were used for identification of *Klebsiella oxytoca* (Table 2).

Table (1): Oligonucleotide primers sequences used for detection of beta-lactamase genes: **Bali *et al.* (2010).**

Target gene	Primers	Primer sequences	References (GenBank no)	Amplicon size (bp)
shv	Forward Reverse	CGCCTGTGTATTATCTCCCT CGAGTAGTCCACCAGATCCT	EF125011	293
tem	Forward Reverse	TTTCGTGTCGCCCTTATTCC ATCGTTGTCAGAAGTAAGTTGG	AB282997	403
ctx-m	Forward Reverse	CGCTGTTGTTAGGAAGTGTG GGCTGGGTGAAGTAAGTGAC	DQ303459	569

Table (2): Oligonucleotide primers used for identification of *Klebsiella oxytoca:* **(Kovtunovych *et al.*, 2003).**

Target gene	Primers Sequences (5'-3')	amplicon size (bp)	References
peh-C	GATACGGAGTATGCCTTTACGGTG	344	Kovtunovych *et al.*,2003
peh-D	TAGCCTTTATCAAGCGGATACTGG		

Isolation of *Klebsiella* species:

In sterile plastic bags, 25 grams of each sample were diluted with 225 ml of 1% sterile peptone water (Merck, Darmstadt, Germany) and homogenized for 10 min using a stomacher (Lab Lemco 400, Seward, Worthington, UK), then 1 ml from each dilutions was plated onto MacConkey agar , trypticase Soya agar and eosin-methylene blue agar **(Brisse *et al.*, 2006)**. All plates were incubated for 24-48 hours at 37 °C and suspected mucoid lactose fermenter colonies were picked up and preserved in semi-solid nutrient agar tubes for further identification. The suspected colonies were identified as *K. oxytoca and K. pneumoniae* morphologically and biochemically according to **Koneman *et al.* (1996).** Results were confirmed by using API 20 E biochemical identification kit.

Antimicrobial susceptibility test:

The disk diffusion technique was applied according to **Finegold and Martin (1982)** using Mueller-Hinton agar (Oxoid), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2014).

Nine different antibacterial sensitivity discs were used for confirmation of resistance of the isolates. The antimicrobial agents tested and their corresponding concentrations were as follows: ampicillin (30 µg), imipenem (10 µg), cefepime (30 µg), cephalothin (30 µg), ceftriaxone (30 µg), cefpodoxime (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), and sulfamethoxazole (25 µg). The results were interpreted in accordance with criteria provided by CLSI (2014).

Polymerase chain reaction:

DNA extraction: DNA extraction was carried out using QIA mp QIAGEN extraction kit

Agarose gel electrophoresis: was applied for separating, identifying and purifying DNA fragments using an agarose concentration appropriate for the size of the DNA fragments to be separated

PCR procedure:

The thermal cycling conditions performed were as follows: 1 cycle of denaturation at 95°C for 4 min; 35 cycles of melting at 95°C for 45 sec, and extension at 72°C for 45 sec.; and a final extension at 72°C for 10 min. (Table 3). Annealing was carried out at various temperatures and times depending on the primer pair used (Table 4).

Table (3): Thermal cycling conditions during PCR (denaturation and extension for all genes)

Step	Temperature	Time	Number of cycles
Initial denaturation	**95°C**	**5 minutes**	**1 cycle**
Denaturation	**95 °C**	**45 seconds**	**35 cycles**
Extension	**72 °C**	**45 seconds**	
Final extension	**72°C**	**10 minutes**	**1 cycle**

Table (4): Temperature and time of annealing in thermal cycling conditions during PCR

Genes	Temperature	Time
peh **gene**	**62°C**	**1 minute**
shv **gene**	**58°C**	**45 seconds**
tem **gene**	**58°C**	**45 seconds**
ctx-m **gene**	**60°C**	**1min**

RESULTS

Isolation of *Klebsiella* species from meat and meat product samples:

As shown in (Table 5), ninety out of the 470 (19.20%) of meat and meat product samples yielded isolates that showed the typical colony characteristics of *Klebsiella* .The isolation rate was slightly higher (20,0%) in meat samples than that (18,5%) in meat products.

Table (5): Results of isolations of *Klebsiella* spp. from meat and meat products

Samples	No. of examined samples	No. of samples positive for *Klebsiella* spp.	%
Meat	**124**	**26**	**20.0**
Meat products	**346**	**64**	**18.5**
Total	**470**	**90**	**19.2**

Prevalence of *K. oxytoca and K. peumoniae* in meat and meat products:

The Prevalence of *K. oxytoca and K. peumoniae* in meat and meat products is depicted in Table (7). In general, the prevalence rate of K. *peumoniae* was higher (11.3%) than that of *K. oxytoca* (7.9%). It is evident that the highest prevalence of *K. peumoniae* was found in luncheon samples (16.0%), followed by imported frozen minced meat (15,0%), basterma (13.9%), sausages (13.0%), local minced meat (12.5%), imported minced meat (8.6%), kofta (8.2%), local meat (8.0%), hot dog (8.1%) and beef burger (7.8%), while all samples of canned beef were negative. On the other hand, the prevalence rate of *K. oxytoca* was highest in local meat samples (12.0%), followed by luncheon samples (11.3%), mported frozen minced meat (10.0%), basterma (9.3%), imported frozen meat (8.6%), local minced meat (8.3%), sausage (6.5%), kofta (5.9%), hot dog (5.4%) and the least was beef burger (3.9%). Also here all samples of canned beef were negative for *K. oxytoca*.

Table (6): Prevalence of *K. oxytoca and K. peumoniae* in meat and meat products

Samples	No. of examined samples	Positive results			
		K. oxytoca		*K. Pneumoniae*	
		No.	%*	No.	%*
Imported frozen minced meat	40	4	10	6	15
Imported frozen meat	35	3	8.6	3	8.6
Local meat	25	3	12	2	8.0
Local minced meat	24	2	8.33	3	12.5
Kofta	34	2	5.9	3	8.2
Sausage	46	3	6.5	6	13.0
Hot dog	37	2	5.4	3	8.1
Canned beef	29	0	0	0	0
Luncheon	106	12	11.3	17	16.0
Basterma	43	4	9.3	6	13.9
Beef burger	51	2	3.9	4	7.8
Total *	470	37	7.9	53	11.3

***The percent was calculated according to the no. of examined samples.**

Results of antibiotic sensitivity testing:

The results presented in (Table 8) show that all isolates were resistant to cephalothin (100%), followed by ampicillin (91.7%), cefpodoxime (75%), cefotaxime (66.7%), sulfamethazole (41.7%), ceftazidime (33.3%), ceftriaxone (16.7%) then the least resistance was observed against imipenem and cefepime (8.3%).

Multiple resistances:

Multiple resistances, i.e. to 3 or more antibiotics, when the isolates were tested against 9 antibiotics, were detected in 91.66. On the other hand, 83.33% of the isolates showed resistance to ≥ 4 antibiotics and 41.66% of the isolates were resistant to ≥ 5 antibiotics.

Table (7): Results of antibiotic resistance of ***K. oxytoca*** and ***K. pneumoniae*** **isolates**

Isolates	Sulfona-mides		β-lactam antibiotics						
	Sulfa-metha-zole	Cepha -lothin	Ceft-azidime	Ceftria x-one	Cefe Pime	Cefpod oxime	Imi-penem	Cefo-taxime	Ampi-cillin
***K.oxytoca*:**									
Basterma	R	R	S	S	S	R	S	S	R
Luncheon	S	R	S	S	R	S	S	R	R
Imported frozen minced meat	S	R	R	S	S	R	S	R	R
Imported frozen meat	R	R	R	R	S	R	S	R	R
Luncheon	S	R	S	S	S	R	S	R	R
***K. pneumoniae*:**									
Luncheon	R	R	S	S	S	R	S	R	R
Beef burger	S	R	R	S	S	R	S	R	R
Local minced meat	S	R	S	S	S	S	S	R	R
Local meat	S	R	S	R	S	R	S	S	R
Hot dog,	S	R	S	S	S	S	S	S	S
Imported frozen minced meat	R	R	R	S	S	R	R	R	R
Basterma	R	R	S	S	S	R	S	S	R
Result %*	41.7	100	33.3	16.7	8.3	75	8.3	66.7	91.7

***% was calculated according to total no. of isolates (12).**

Results of PCR testing of Klebsiella isolates

Detection of identification genes of *K. oxytoca*:

Photo (1): PCR analysis for detection of *peh* gene (gene of identification of *K. oxytoca*)

M : Marker ; Lane 1,2,3,4,5 samples were positive and had molecular weight 344bp Lane (1) *K. oxytoca* isolated from basterma, Lane (2) *K. oxytoca* isolated from luncheon, Lane (3) *K. oxytoca* isolated from imported frozen minced meat, Lane (4) *K. oxytoca* isolated from imported frozen meat, Lane (5) *K. oxytoca* isolated from luncheon, Lane (6,7,8,9,10,11,12) *K. pneumoniae* isolated from luncheon, beef burger, local minced meat,local meat, hot dog, imported frozen minced meat, and basterma are negative.

Detection of β-lactam resistance genes:

Table (8) and Photos (2, 3, 4) demonstrate the high prevalence of *shv*, *tem*, *ctx-m* β-lactam resistance genes, which were detected in 12 (100%), 11 (91.7%) and 9 (75%) of the isolates respectively. *tem* gene was detected in *K. pneumoniae* isolates recovered from all types of meat and meat products except basterma. In addition, the *ctx-m* gene was absent in *K. oxytoca* isolates recovered from basterma, while it was absent in *K. pneumoniae* isolates obtained from meat and minced meat.

Table (9): The result of the PCR of β-lactam resistance genes

Isolates	*shv*	*tem*	*ctx-m*
***K. oxytoca*:**			
Basterma	+	+	-
Luncheon	+	+	+
Imported frozen minced meat	+	+	+
Imported frozen meat	+	+	+
Luncheon	+	+	+
***K. pneumoniae*:**			
Luncheon	+	+	+
Beef burger	+	+	+
Local minced meat	+	+	+
Local meat	+	+	-
Hot dog,	+	+	-
Imported frozen minced meat	+	+	+
Basterma	+	-	+
% of positive isolates	**12(100%)**	**11(91.66%)**	**9 (75%)**

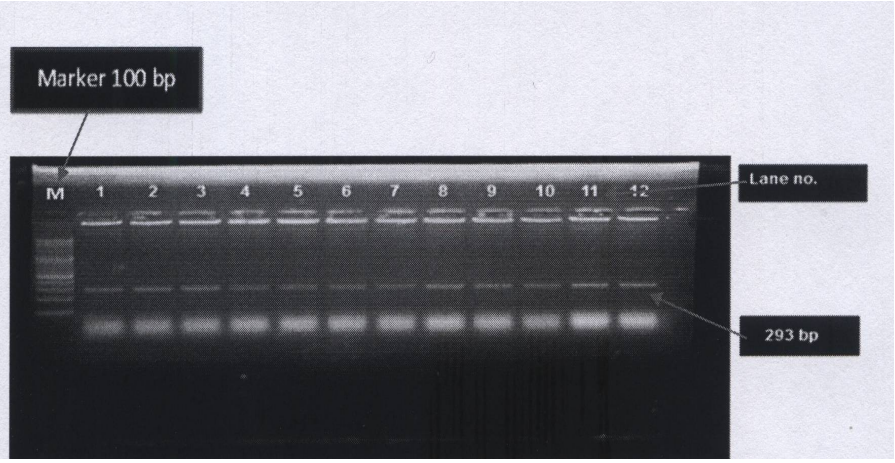

Photo (2): PCR analysis for detection of *shv* gene M : Marker ;from Lane 1 to 12 samples were positive and had molecular weight 293bpLane (1) *K. oxytoca* isolated from basterma, Lane (2) *K. oxytoca* isolated from luncheon, Lane (3) *K. oxytoca* isolated from imported frozen minced meat, Lane (4) *K. oxytoca*

isolated from imported frozen meat, Lane (5) *K. oxytoca* isolated from luncheon, Lane (6) *K. pneumoniae* isolated from luncheon, Lane (7) *K. pneumoniae* isolated from beef burger, Lane (8) *K. pneumoniae* isolated from local minced meat, Lane (9) *K. pneumoniae* isolated from local meat, Lane (10) *K. pneumoniae* isolated from hot dog, Lane (11) *K. pneumoniae* isolated from imported frozen minced meat, Lane (12) *K. pneumoniae* isolated from basterma.

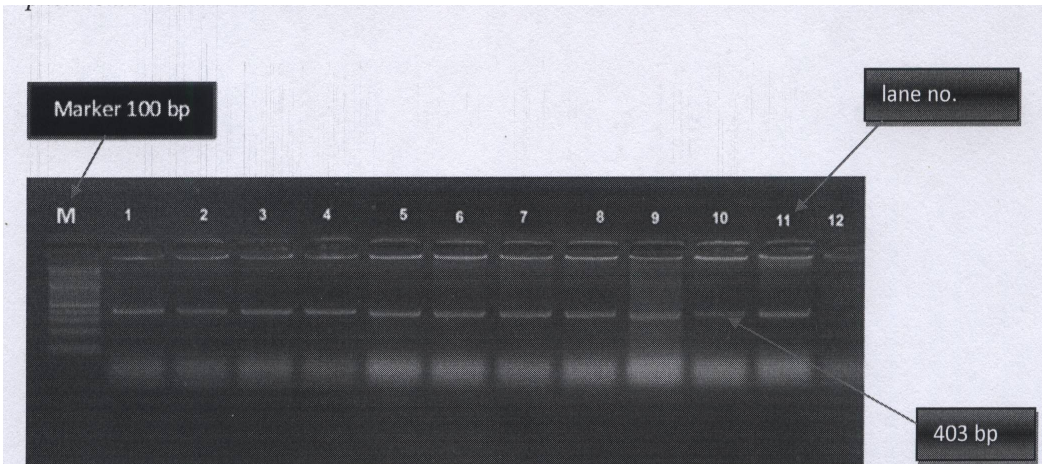

Photo (3): PCR analysis for detection of *tem* gene M : Marker ; From lane 1 to 11 samples were positive and had molecular weight 403bp, Lane (1) *K. oxytoca* isolated from basterma, Lane (2) *K. oxytoca* isolated from luncheon, Lane (3) *K. oxytoca* isolated from imported frozen minced meat, Lane (4) *K. oxytoca* isolated from imported frozen meat, Lane (5) *K. oxytoca* isolated from Luncheon,, Lane (6) *K. pneumoniae* isolated from luncheon, Lane (7) *K. pneumoniae* isolated from beef burger, Lane (8) *K. pneumoniae* isolated from local minced meat, Lane (9) *K. pneumoniae* isolated from local meat, Lane (10) *K. pneumoniae* isolated from hot dog, Lane (11) *K. pneumoniae* isolated from imported frozen minced meat and Lane (12) *K. pneumoniae* isolated from basterma was negative.

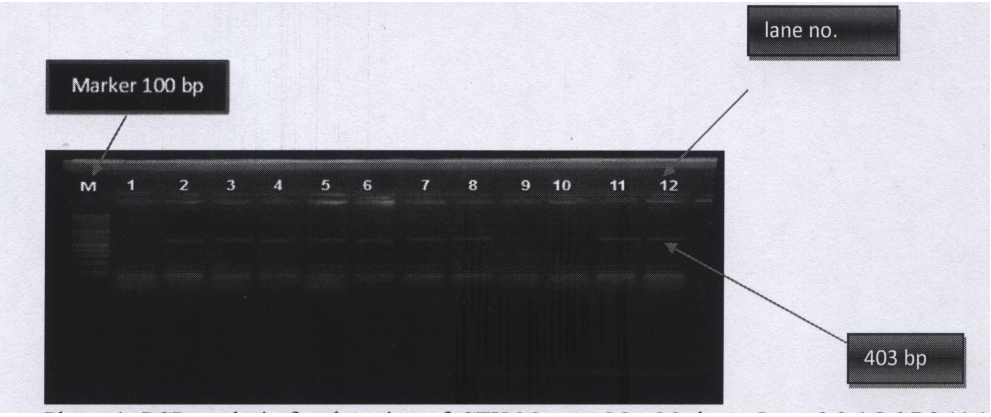

Photo (4): PCR analysis for detection of *ctx-m* gene M : Marker ; Lane 2,3,4,5,6,7,8,11,12 samples were positive and had molecular weight 569bp**,** lane (2) *K. oxytoca* isolated from luncheon, Lane (3) *K. oxytoca* isolated from imported frozen minced meat, Lane (4) *K. oxytoca* isolated from imported frozen meat, Lane (5) *K. oxytoca* isolated from luncheon, Lane (6)) *K. pneumoniae* isolated from luncheon, Lane (7) *K. pneumoniae* isolated from beef burger, Lane (8) *K. pneumoniae* isolated from local minced meat, Lane Lane (11) *K. pneumoniae* isolated from imported frozen minced meat, Lane (12) *K. pneumoniae* isolated from basterma. Lane (1) *K. oxytoca* isolated from basterma and Lane (9, 10) *K. pneumoniae* isolated from local meat, hot dog are negative.

DISCUSSION

Klebsiella spp. are ubiquitous in nature and have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horses, or swine, on which they colonize. The gastrointestinal tract and the hands of personnel were reported as principal reservoirs of *Klebsiella* spp. The presence of *K. pneumonia*e in street foods indicate the potential faecal contamination, possible cross-contamination between food handlers, food preparation, surfaces and the food itself **(Gundogan and Yakar, 2007 and Haryani *et al.*, 2007).** In the present study, the prevalence of *K. oxytoca* and *K. pneumoniae* in meat and meat products indicates a higher isolation of *K. oxytoca* in meat (9.7%) than in meat products (7.9%), while *K. pneumoniae* the rate was the same in meat and meat products (11.3%). The isolation rate was higher in imported minced meat (10% for *K. oxytoca* and 15% for *K. pneumoniae*) in comparison with the local minced meat (*K. oxytoca* 8.3% and

K.pneumoniae 12.5%). All samples of canned beef were negative. The highest isolation rate among the meat product samples was from luncheon (11.3% *K. oxytoca* and 16.0% *K .pneumoniae*) and basterma (9.3% *K. oxytoca* and 13.9% *K .pneumoniae*) and the lowest was in beef burger **(**3.9% for *K. oxytoca* and7.8% *K. pneumoniae*)**.** These results agree with those reported by **Mohammed (2011)**, who isolated *Klebsiella* spp from 8 samples (10.7%) from meat products. **Abdelomonem *et al.* (2009)** isolated *Klebsiella* spp. from 33. 3% of meat products samples. The antibiotic resistance of *K. oxytoca* and *K. pneumoniae* isolates against the tested antimicrobial agents was 100 % against cephalothin followed by ampicillin (91.7%), cefpodoxime (75%), cefotaxime (66.7%), sulfamethoxazole (41.6%), ceftazidime (33. 3%), ceftriaxone (16.7%) and finally 8.3% for cefepime and imipenem. These results coincide with observation reported by **Subha and Ananthan (2002)**, who found that 95% of the *K. pneumoniae* isolates showed resistance to ceftazidime, cefotaxime, ceftriaxone. **Shawkat and Nabil (1999) and Haryani *et al.* (2007)** reported that *K. pneumoniae* isolates were resistant to ampicillin (100%), while **Brisse and Duijkeren (2005)** found that 100 *Klebsiella* animal clinical isolates were resistant against ampicillin (99%) and trimethoprim-sulfamethoxazole. Cephalosporins are important class of antibacterial agents in use for both humans and animals. The use of cephalosporins in food-producing animals could be selective factor for the appearance of ESBL-producing and multiple-antimicrobial-resistant bacteria in such animals **Cavaco *et al*. (2008)**. **Al-Agamy *et al.* (2009a)** found that, the resistance rate of ESBL-producing *K. pneumoniae* to cefotaxime and ceftazidime were 97% and 95%, respectively. On the other hand, **Shigemoto *et al.* (2013)** reported that 21 *Klebsiella* isolates from Japan showed susceptibility or intermediate resistance to imipenem and 5 isolates, 2 *Klebsiella oxytoca* and 3 *K. pneumoniae*, were resistant to almost all of the β-lactam antibiotics except imipenem. Also **Newire *et al.* (2013)** found that all isolates of *K. pneumoniae* isolated from nosocomial were resistant to ceftazidime and cefotaxime. Extended-spectrum-β -lactamases producing genes were usually encoded by a plasmid carrying multiple genes conferring resistance to other antimicrobial agents. This further complicates the clinical treatment of such bacterial infections. Therefore, it is important to monitor closely extended-spectrum-β -lactamases producing strains and to prevent their spread. In the present work, the β-lactam resistant gene were identified by the use of the specific primers of these genes (*shv* gene had molecular weight 293 bp, *tem* gene had

molecular weight 403 bp and *ctx-m* gene had molecular weight 569 bp). It was observed that (100%) of the tested isolates had *shv* gene, while tem gene was detected in (91.7%) of the tested isolates and *ctx-m* gene was detected in (75%) of the tested isolates. These findings are in agreement with those of **Al-Agamy (2013**). He detected a high prevalence of *shv* (100%) and *ctx-m* (100%) and low frequency of *tem* (40%) in extended-spectrum-β-lactamases producing *K. pneumonia*e isolates. However, it disagrees with the results obtained by **Jain and Mondal (2008),** as they mentioned that in ESBL producing *Klebsiella* spp. isolates tested by them, 17 (26.5%) had both *tem* and *shv* genes, 31 (48.4%) had *tem* alone and 13 (20.3%) had *shv* gene alone. Three (4.6%) ESBL positive isolates were negative for both *tem* and *shv*. The *Klebsiella* spp. isolates with both *tem* and *shv* genes were highly resistant to all antibiotics used in the test.

Al-Agamy *et al.* (2009b) determined that, the ESBL-producing *K. pneumoniae* isolates were PCR positive for *shv*, *tem* and *ctx-m* β-lactamase genes with prevalence rates of 97.3%, 84.1% and 34.1%, respectively.

Recommendation:

It is clear that the increasing prevalence of resistance in the isolates from animal origin may have important therapeutic implications. Thus, monitoring of ESBL-producing *Klebsiella* should be continued at various levels (animals, human, and environment), with continuing investigating the factors that contribute to their selection and dissemination. Antibiotics should be given after making sensitivity test to the isolated organisms and in recommended dose, route and duration of usage.

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