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**RESEARCH ARTICLE**

# OCCURRENCE OF EXTENDED SPECTRUM BETA LACTAMASE RESISTANT *KLEBSIELLA PNEUMONIAE* (*ESBL*) IN RAW WHITE SOFT CHEESE AND ITS WHEY FROM BAGHDAD

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**ABSTRACT**

Carbapenemases producing or Extended Spectrum Beta Lactamase Resistant *Klebsiellapneumoniae* (*ESBL*/*KPC*) strains are pro-growing threat. This research focused on isolation and Identification of *K.pneumoniae* especially *ESBL* producers from raw white soft cheese and its whey made from Cows raw milk in Baghdad. Study design including collection and processing of ninetysoft cheese and its whey samples (forty five samples for each type: soft cheese & whey) from regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya (thirty samples from each region: fifteen samples for each type) from December (2016) to February (2017), in which they collected and processed according to modified dairy microbiological methodology in veterinary public health laboratory by McConkey agar, Electronic RapID™ ONE (4 hours) biochemical panel micro-tubes strep identification system compendium with reference colors chart and online confirmation microcodes data base software, and gold standard double staining technique, Microtiter Plate Assay for biofilm formation with methylene blue and safranin dyes. Antibiotics Susceptibility Pattern by Kirby-Bauer technique or disk diffusion method was proceed according to instructions of clinical laboratory standards institute (*CLSI*) or national committee for clinical laboratory standards (*NCCLS*) by using a Muller-Hinton agar and McFarland opacity tubes for checking resistance profile of isolates. Double diffusion inhibition technique or Oxoid Cefpodoxime Combination Kit determined *ESBL* resistance activity. Data were analyzed for significant differences by statistical package for social sciences software (*IBM SPSS*) in which a Chi-square was used. The results showed detection of thirteen strains of phenotypically indole negative *K.pneumoniae* out of ninety samples (14.44%): ten strains (11.11%) from Abu-Ghraib: nine (10%) from soft cheese and one (1.11%)from its why, two strain (2.22%) from Al-Fudhaliyah and one (1.11%) strain from Al-Sadrya. *ESBL* producers were noticed only from Abu-Ghraib region, in which they detected from seven (7.77%) samples: six (6.66%) strains from soft cheese and one (1.11%) from its whey. In conclusion, data showed contamination of raw white soft cheese and its whey in Baghdad with *K. pneumoniae*, in which *ESBL* producers were noticed, thus we recommend monitoring of soft cheese production cycles, handlers, cheese makers, soft cheese containers, environment, infected & carrier individuals, transportation and storage stages through application of sanitation practices during cheese processing and hazard analysis critical control points (*HACCP*) strategies to overcome or reduce these public health problems.



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## INTRODUCTION

Multidrug-resistant *K. pneumoniae* harboring New Delhi metallo-β-lactamase (NDM-1) was detected in-patient who had returned to Canada from India. *K. pneumoniae* has been associated with different types of infections as emergence of multi-drug resistant strains (Sikarwar and Batra, 2011; Rupp and Fey, 2003). Epidemic strains of multi cephalosporin generations resistant *K .pneumoniae* cause serious nosocomial and community acquired infections that are hard to eliminate. *K. pneumoniae* strains were plasmid mediated TEM or SHV ancestries that are transferable from strain to strain and between bacterial species (Sikarwar and Batra, 2011; Rupp and Fey, 2003). Many ESBL producing strains also express AmpC β-lactamases that might be co-transferred with plasmids mediating aminoglycoside resistance. Multi slime layers complex or biofilm clouds might be primarily the reason of antibiotics resistance. Although β-lactamase inhibitors such as clavulanic acid inhibit *K. pneumoniaein vitro*, the activity of these inhibitors is influenced by the bacterial inoculum, dose administration regimen and genetic type of ESBL strain. Currently, carbapenems are considered as the drugs of choice for treatment of infections caused by ESBL microorganisms. Unfortunately, they have been associated with the emergence of carbapenems resistant strains. Emergence of Multi-drug resistant strains is associated with different resistant strategies that reduceand modify the effects of antibiotics profile (Sikarwar and Batra, 2011; Rupp and Fey, 2003; Thenmozhi *et al*., 2014). Routine antibiotic susceptibility testing failed to identify *KPC* producers. Combination therapy might be preferable to control *KPC* infections in immediate future (Swathi *et al*., 2016). The *KPCs* have become endemic in many countries but there is no optimal treatment recommendation available for bacteria expressing *KPCs*. Carbapenems are broad-spectrum antibiotics similar to β-lactams. Resistance

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emerged through alterations in drug targets like penicillin binding proteins (*PBPs*), porin loss and upregulation of efflux pumps and expression of carbapenemases. Carbapenemases are divided into two major types: metallo β-lactamases (Class B) containing zinc at the active site (*NDM-1*) whereas serine β-lactamases (Classes A, C and D) containing serine at the active site. The *K. pneumonia* Carbapenemases constitute the class A (Swathi *et al*., 2016).

Initially *KPC* strains were restricted to sporadic outbreaks, but the situation became difficult because of global frequency and distribution in both developed and developing countries (Chen *et al*., 2012; Virgincar *et al*., 2011; Endimiani *et al*., 2009; Jacome *et al*., 2012). One of the most popular and traditional dairy food in Iraq is soft cheese especially white sweet types due to social nature. As we know, that processed soft cheese made from raw and pasteurized Cow's milk harboring many foodborne pathogens especially those causing food poisoning in man, due to its sequestering spongiform nature, water content or water activity and handlers or cheese makers that processing cheese under poor sanitary environment or from unhygienic animals from poor unclean raw milk or been actively infected or passively carriers with multidrug resistant pathogens especially in rural areas in Iraq, as well as its whey and containers like raw milk could harboring the persistent and resident infectious foci of these pathogens. All these events and others about the nature of soft cheese in Iraqi environment with low information about quality of raw madesoft cheese as well as environmental hygienic conditions of milk producing animals and their milk production and processing cycles under these circumstances, and obscure information about dominance of *K. pneumonia*e pointing to inquiry of the existence, spreading and rate of these contaminant in raw white soft cheese and its whey samples from regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya.

## MATERIALS AND METHODS

### Collection & Processing of Samples

Study design including collection and processing of ninetysoft cheese and its whey samples (forty five samples for each type: soft cheese & whey) from regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya (thirty samples from each region: fifteen samples for each type) during period December (2016) to February (2017), in which they collected and processed according to modified dairy microbiological methodology and bacteriological analytic manualin veterinary public health laboratory (British Standards Institution, 1984; Marshall, 1993; Food and Drug Administration, 2000; Quinn *et al*., 2004; Food Safety and Inspection Services, 2013; Bacteriological Analytical Manual, 2017). Samples were collected from raw soft cheese and its whey containers (sample made up and processed from pooled raw milk of cows, thermally unprocessed whole organoleptic raw soft cheese made directly from unpasteurized raw milk) via cleanbags carefully to minimize contamination and processed adequately until transportation to work lab by ice box, then refrigerated at 4 $^{©}$C as critical control point in isolation and identification procedure of psychrotrophic *K. pneumonia*e.

### Isolation & Identification Procedure

All samples were processed according to food microbiological techniques in which they refrigerated at 4 $^{©}$C for 48 hours for resuscitation of psychrotrophic *K. pneumonia*e, then soft cheese sample were emulsified by stomacher with 2% buffered trisodium citrateinside double (500 ml) nondurable clean bags to disintegrate it in to small lobules that hide the pathogen. Processed soft cheese samples were diluted and inoculatedin buffered tryptone soya yeast extract broth (one part sample (10 gm) to nine part diluent (90 ml) and incubated at 37 $^{©}$C for 24 hours, then inoculated in McConkey agar by loop (dilution inoculation) in three replicates for each sample, then incubated at 37 $^{©}$C for 48 hours (British Standards Institution, 1984; Marshall, 1993; Food and Drug Administration, 2000; Quinn *et al*., 2004; Food Safety and Inspection Services, 2013; Bacteriological Analytical Manual, 2017). Thoroughly mixed whey samples with stomacher were processed as above but without addition of sodium citrate. Pure large mucoid and capsulated pink colonies with viscous threads were picked up and recultured in tryptone soya yeast extract brothto proliferate and revive stressed isolates, then cultured in tryptone soya yeast extract agar for further identification. Gram stain and capsule stainproceeds. Electronic RapID™ ONE(4 hours) biochemical panel micro-tubes strep identification system compendium with reference colors chart and online confirmation microcodes data base software was used for identification procedure according to company leaflet instructions (Oxoid – Remel, 2016).

### Biofilm Formation Assay

Double staining technique, Microtiter Plate Assay with methylene blue and safranin dyes was used for detection of biofilm formation and secretion. Quantitavely and Qualitative detection of slime producer strains was determined by culturing the isolates on modified Tryptone Soy Yeast Extract Broth (TSBYE) using adherence assay on large U-shape 24 well tissue culture plates as described previously by Christensen *et al*. (Christensen *et al*., 1985; O'Toole, 2011; Welch *et al*., 2012). An overnight culture grown in TSBYE at 37$^{©}$C was transferred and diluted in microtiter plate as 0.1ml/0.5ml freshly prepared TSBYE inoculated for each well. Each isolate was tested in triplicate. Wells with sterile TSBYE alone was served as controls. The plates were incubated for 24 hours at 37°C. Furthermore, the culture was removed and plates were washed three times with phosphate-buffered saline to remove non-adherent cells and dried in an inverted position. Adherent biofilm was fixed with 2% sodium acetate and was stained with of 10% crystal violet and safranin for 5 min. Then, unbound stain was removed and the wells were washed three times with PBS. Plates were settled 2-3 hours for dryness then stained layers and dots of biofilm in bottom and around internal rims of wells were photographed, measured and scored according to the degree of formation, type of stain and type of isolate.

### Antibiotics Susceptibility Pattern (Resistance Profile)

A Kirby-Bauer technique or disk diffusion method was dependent according to instructions of clinical laboratory standards institute (*CLSI*) or national committee for clinical laboratory standards (*NCCLS*) by using a Muller-Hinton agar and McFarland opacity tubes (Bauer *et al*., 1966; Clinical and Laboratory Standards Institute, 2009a; Clinical and Laboratory Standards Institute, 2009b; Lalitha, 2004; Jorgensen and Ferraro, 2009; Korgenski and Daly, 1998). A test procedure was done by selecting well-isolated (4-5) colonies of *K. pneumonia* from freshly inoculated overnight TSAYE, touched tops of these colonies by a loop then, transferred to freshly

prepared (4-5) ml TSBYE tubes and incubated for 2 hr. at 37 $^{©}$C in order to reach a standard 0.5 opacity of McFarland tubes or approximately $10^4$-$10^5$ cfu/ml standard inoculum broth. Preparation of freshly agar plate's cultures of Muller-Hinton then dried in incubator before testing procedure. A sterile cotton swab was dipped into the adjusted suspension and rotated several times and pressed firmly on the inside wall of the tube above the fluid level for removing excess inoculum from the swab. Streaking the surfaces of Muller-Hinton agars (4-5) times with the rim by a swabs then left inoculated agars for (10-15) minutes to absorb the inoculum before applying selected antimicrobial disks by pressing down to ensure complete contact with the agar surface and distributed evenly. The plates were inverted and placed in an incubator at 37 $^{©}$C for (18-24) hrs. Then reading the plates and interpretation the results (Bauer *et al*., 1966; Clinical and Laboratory Standards Institute, 2009a; Clinical and Laboratory Standards Institute, 2009b; Lalitha, 2004; Jorgensen and Ferraro, 2009; Korgenski and Daly, 1998). If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter. The diameter of the zone was related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted using the criteria published by *CLSI*. The results were qualitative as susceptible, intermediate or resistant isolate derived from the test rather than minimum inhibitory concentration (*MIC*).

### *ESBL* Combination Disk Technique

Extended spectrum beta lactamase resistance activity was determined in food laboratory for study isolates by double diffusion inhibition technique or Oxoid Cefpodoxime Combination Kit (Oxoid – Remel, 2016). Combination discs were a blend of cephalosporin and clavulanic acid on a single disc, which are, used in conjugation with a plain cephalosporin for *in vitro* detection of *ESBLs* strains that do not produce inducible *AmpC* enzymes. The kit contains the following: Cefpodoxime/ clavulanic acid (CD01) 10/1µg & Cefpodoxime (CPD10) 10µg. The isolates were tested by comparing the inhibition zones given by cefpodoxime (10-mg) and cefpodoxime-plus-clavulanate (10- plus 1-mg) disks. The presence of clavulanate enlarged the zones for all of *ESBL*-producing *klebsiellae* by ≥ 5 mm, whereas zones for cefpodoxime-susceptible isolates and cefpodoxime-resistant isolates with *AmpC* and *K1* β-lactamases were enlarged by ≤ 1 mm. Good discrimination was achieved with either the *NCCLS* (*CLSI*) or British Society for Antimicrobial Chemotherapy (*BSAC* Standardized Disc Sensitivity Testing Method) (Carter *et al*., 2000; De Gheldre *et al*., 2003; Jarlier *et al*., 1988). Combination discs should be used by qualified personnel trained to handle category 2 resistant pathogens, and be competent in basic microbiological techniques including antibiotic susceptibility testing. The discs need to be placed on sensitivity media (Muller-Hinton agars) with sufficient space between the discs to allow the formation of clearly defined zones of inhibition and combination of them. A freshly prepared standardized inoculum 0.5 McFarland from each isolate on TSBYE was used in test procedure ($10^4$-$10^5$ cfu/ml). Ceftazidime (CAZ) (30-mg) disks (Oxoid) were tested in parallel as a control (Jarlier *et al*., 1988). Zone diameters were measured to the nearest millimeter. A difference of ≥ 5 mm between the zones of the CD01 (10- plus 1-mg) and CPD (10-mg) disks was taken to indicate *ESBL* production, as advocated by the manufacturer. The zones of the CAZ (30-mg) and CPD (10-mg) disks additionally were compared against the susceptibility criteria of the *NCCLS* and *BSAC* for predicting ESBL production (National Committee for Clinical Laboratory Standards, 2000).

### Statistical Analysis

Chi-square (æ $^2$) analysis used for checking significant differences among data, through statistical package for social sciences software (IBM-SPSS, 2016).

## RESULTS AND DISCUSSION

### Isolation & Identification Profile

The results showed detection of thirteen strains of phenotypically indole negative *K.pneumoniae* out of ninety samples (14.44%): ten strains (11.11%) from Abu-Ghraib: nine (10%) from soft cheese and one (1.11%) from its why, two strain (2.22%) from Al-Fudhaliyah, and one (1.11%) strain from Al-Sadrya. *ESBL* strains were noticed only from Abu-Ghraib region, in which they detected from seven (7.77%) samples: six (6.66%) strains from soft cheese and one (1.11%) from its whey. Detection Profile and isolation percentages illustrated in Tables (1,2&3):

**Table 1. Isolation of *K. pneumoniae* from raw white soft cheese**

| Region | Number of Samples | Isolation % (15) | Isolation % (45) |
|---|---|---|---|
| Abu-Ghraib | 15 | 9 (60%) $^{A*}$ | 9 (20%) $^{A*}$ |
| Al-Fudhaliyah | 15 | 2 (13.4%) $^{B}$ | 2 (4.5%) $^{B}$ |
| Al-Sadrya | 15 | 1 (6.7%) $^{B}$ | 1 (2.3%) $^{B}$ |
| Total | 45 | 12 (80.1%) | 12 (26.8%) |

*: Indicate highest isolation ratio from Abu-Ghraib.
A,B: Indicate significant differences (æ$^2$) vertically at level (P≤0.05).

**Table 2. Isolation of *K. pneumoniae* from whey of raw white soft cheese**

| Region | Number of Samples | Isolation % (15) | Isolation % (45) |
|---|---|---|---|
| Abu-Ghraib | 15 | 1 (6.7) $^{A*}$ | 1 (2.3%) $^{A*}$ |
| Al-Fudhaliyah | 15 | None $^{B}$ | None $^{B}$ |
| Al-Sadrya | 15 | None $^{B}$ | None $^{B}$ |
| Total | 45 | 1 (6.7%) | 1 (2.3%) |

*: Indicate highest isolation ratio from Abu-Ghraib.
A,B: Indicate significant differences (æ$^2$) vertically at level (P≤0.05).

**Table 3. Isolation of *K. pneumoniae* from raw white soft cheese with its whey**

| Region | Number of Samples | Isolation % (30) | Isolation % (90) |
|---|---|---|---|
| Abu-Ghraib | 30 | 10 (33.4%) $^{A*}$ | 10 (11.11%) $^{A*}$ |
| Al-Fudhaliyah | 30 | 2 (6.66%) $^{B}$ | 2 (2.22%) $^{B}$ |
| Al-Sadrya | 30 | 1 (3.33%) $^{B}$ | 1 (1.11%) $^{B}$ |
| Over all Total | 90 | 13 (43.39%) | 13 (14.44%) |

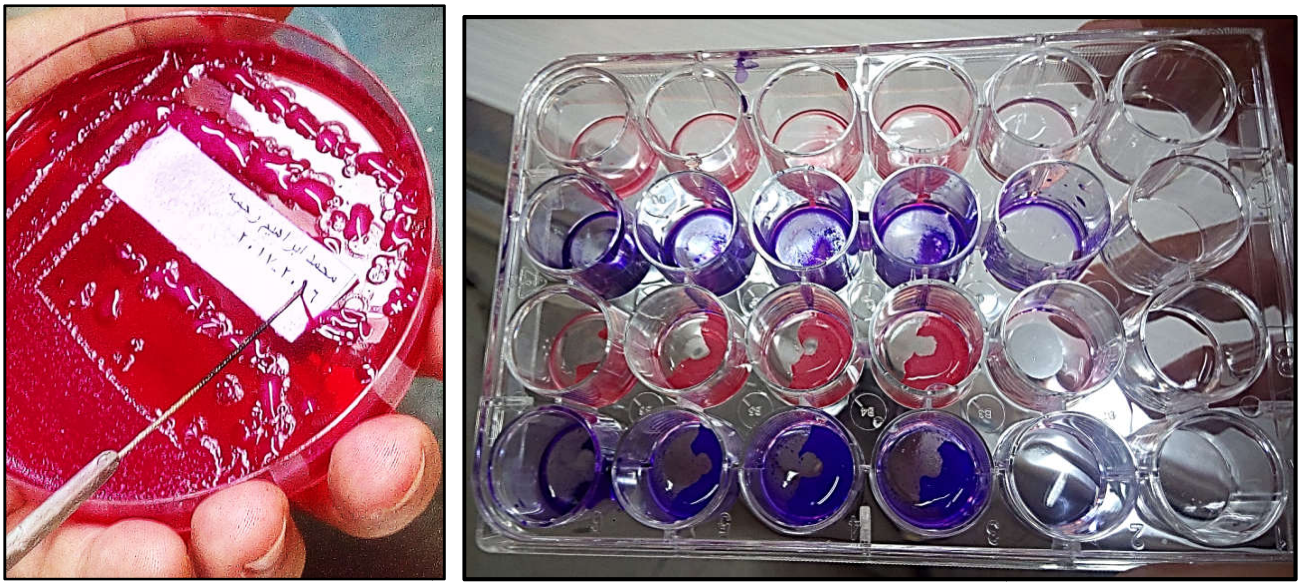
*: Indicate highest isolation ratio from Abu-Ghraib.
A,B: Indicate significant differences (æ$^2$) vertically at level (P≤0.05).

Microbial quality and quantity of milk and dairy products depends on hygienic measurements during milk production and dairies manufacturing. The examination for the microbial load of specific microorganisms isan vital part of any quality assurance plan and that might be applied to a number of areas including raw materials, samples, finished products, or environmental (Bramley and McKinnon, 1990; Anonimus, 1994; Torkar and Teger, 2006; El-Sukhon, 2003).

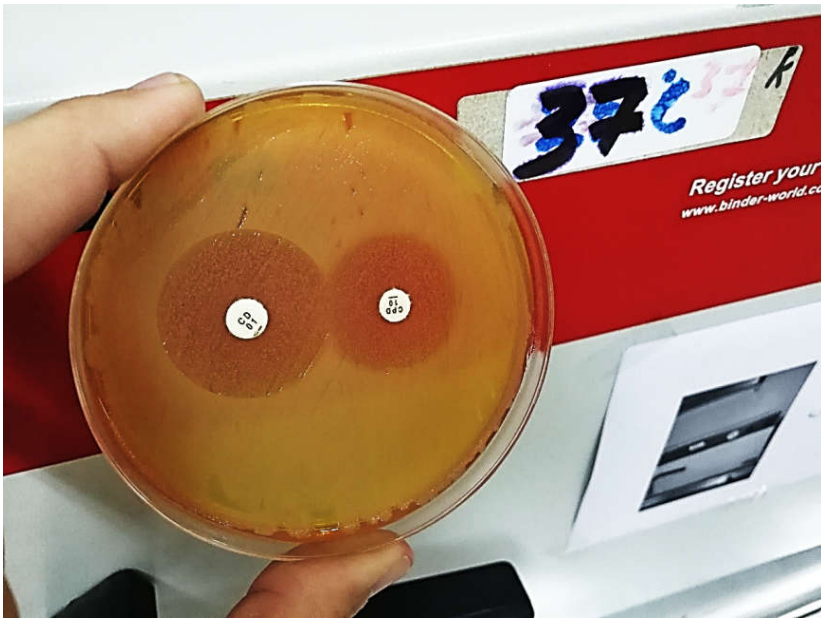
**Table 4. Pattern of Resistance, Intermediate and Susceptible isolates of *K. pneumoniae* (total 13 isolates) from raw white soft cheese and its whey in Baghdad**

| Antibiotics | Concentration | Resistance % | Intermediate % | Susceptible % |
|---|---|---|---|---|
| Penicillin G (P) | 10 IU | 13(100)$^{Aa}$ | None (0)$^{Bb}$ | None (0)$^{Bb}$ |
| Ampicillin (AM) | 10 µg | 13 (100)$^{Aa}$ | None (0)$^{Bb}$ | None (0)$^{Bb}$ |
| Cephalothine (KF) | 30 µg | 13 (100)$^{Aa}$ | None (0)$^{Bb}$ | None (0)$^{Bb}$ |
| Cefotaxime (CTX) | 30 µg | 13 (100)$^{Ac}$ | None (0)$^{Bb}$ | None (0)$^{Bb}$ |
| Aztreonam (AZ) | 30 µg | 12(92.3)$^{Ab}$ | 1 (7.7)$^{Ab}$ | None (0)$^{Bc}$ |
| Imipenem (IMI) | 10 µg | 12(92.3)$^{Aa}$ | 1 (7.7)$^{Ab}$ | None (0)$^{Bc}$ |
| Meropenem (MEM) | 10 µg | 12(92.3)$^{Aa}$ | 2 (15.4)$^{Ab}$ | None (0)$^{Bc}$ |
| Ciprofloxacin (CIP) | 5 µg | 13 (100)$^{Ac}$ | None (0)$^{Bb}$ | None (0)$^{Bb}$ |
| Doxycycline (DXT) | 30 µg | 12(92.3)$^{Aa}$ | 1 (7.7)$^{Ab}$ | None (0)$^{Bc}$ |
| Trimethoprim/Sulfamethoxazole (TS) | (1.25/23.75) µg | 12(92.3)$^{Aa}$ | 1 (7.7)$^{Ab}$ | None (0)$^{Bc}$ |
| Clindamycin (CD) | 2 µg | 13 (100)$^{Ac}$ | None (0)$^{Bb}$ | None (0)$^{Bb}$ |
| Chloramphenicol (C) | 30 µg | 11(84.6)$^{Ba}$ | 2 (15.4)$^{Ab}$ | None (0)$^{Bc}$ |
| Naldixic acid (NA) | 30 µg | 12(92.3)$^{Aa}$ | 1 (7.7)$^{Ab}$ | None (0)$^{Bc}$ |

A,B: Indicate significant differences ($æ^2$) among selected antibiotics for total isolates (20) vertically at level (P≤0.05).
A,b,c: Indicate significant differences ($æ^2$) among total isolates (20) for selected antibiotic horizontally at level (P≤0.05).



**Photograph (1): Colonies of Extended Spectrum Beta Lactamase (ESBL) Resistant K. pneumoniae (KPC strain) on McConkey agar after 48 hrs. at 37 ©C as large mucoid & pink colonies (lactose fermenter) with biofilm formation texture. Biofilm formation on modified microtiter plate assay with double staining technique (methylene blue & safranin). Noticed deposited mucous layers & dots on bottom & around microtiter tubes**




**Photograph (2): *ESBL* producer variants isolated from soft cheese and its whey from Abu-Ghraib region in Iraq, and detected in Cefpodoxime combination disk on Muller-Hinton agar**

Large mucoid pink colonies with viscous threads on McConkey agar with *ERIC* panel aid in confirmation of and indole negative *K. pneumonia* strains from soft cheese samples and its whey from regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya. Isolation profile indicate presence of infectious foci of this pathogen in these areas due to poor sanitary conditions during and after cheese production as well as climatic conditions and inadequate management practices for milk producing animals and milk cans as well as infective individuals and carriers with vectors and other interconnected factors and stressors encourage epidemiological distribution and frequency pattern of these emerging opportunists. Contamination and pollution can occurs at any stage of milk production, handling, transportation and storage. Cheese cans and containers might contains resident adherent biofilm infectious foci of *K. pneumonia* in the bottom, as well as environmental conditions during production, handling, transportation and storage of cheese may facilitate spreading of these multidrug resistant pathogens. Subclinical infected animals' especially subclinical mastitis might become source of infection. Thermally untreated raw milk and raw soft cheese made from unpasteurized raw milk samples were dependent in this study, as well as ripening of raw soft cheese under these situations might harboring and transfer these food borne pathogenies, and that one of the important causes about presence of *K. pneumonia* in them. Risk factors of these food borne pathogens were evident through transportation from livestock to community and nosocomial relationship that facilitate distribution of biofilm producing and multidrug resistant pathogens in Iraqi environment, but actually in low number and cases but remain dangerous.

**Biofilm formation**

Gold standard microtiter tissue culture assay with double staining technique by methylene blue and safranin dyes plus McConkey agar results (large viscous mucoid pink colonies) obviously indicate biofilm formation and secretion. Red and blue adherent layers and dots of biofilm around and in the bottom of microtiter plate tubes indicate presence of biofilm producing isolates as in photograph (1): Photograph (1): Colonies of Extended Spectrum Beta Lactamase (*ESBL*) Resistant *K. pneumoniae* (*KPC* strain) on McConkey agar after 48 hrs. at 37 ©C as large mucoid & pink colonies (lactose fermenter) with biofilm formation texture.Biofilm formation on modified microtiter plate assay with double staining technique (methylene blue & safranin). Noticed deposited mucous layers & dots on bottom & around microtiter tubes. Biofilm producing and multidrug resistant *K. pneumonia* isolates detection was another very important and dangerous risk evident in this study because of persistent source of contamination and pollution of environment, peoples, animals, foods, feeds, etc. from these infectious foci and active and passive carriers. Gold standard double staining technique, Microtiter Plate Assay for biofilm formation with sensitive methylene blue and safranin dyes vitally assist in detection of biofilm producing isolates especially those owing resistance profile to Cefpodoxime/clavulanic acid. Production of biofilm or slim exopolysaccharides protect the isolates from harsh conditions during their life cycle and supply new virulent strains in environment. Genetic makeup and plasmids mediated of these strains may need more interpretation along with the restudy the relationship among environment, milk producing animals and handlers in distribution of these infectious fociin regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya. Risk of biofilm multidrug resistant strains incriminate in difficult to treatment cases of pneumonia, urinary tract infections, etc.

**Antibiotic Susceptibility Pattern**

Resistance Profile of biofilm producing isolates were evident for selected antimicrobials and interpreted according to tables of *CLSI* (2009) as indicated in Table (4):

Genetic bulk of isolate with environment of sample causing antibiotic resistance profile might be partially due to formation of capsular polysaccharides and slime complexthat prevent or reduce the effect of selected antibiotics *in vitro*as noticed in this study. The ability to resist antibiotics may reflect the inelegant strategy and behavior of *K. pneumoniae*to overcome, modulating and buffering environmental and inoculation conditions according to their life cycle *in vivo* and *in vitro*. Risk of biofilm multidrug resistant strains incriminate in difficult to treatment cases of pneumonia, urinary tract infections, etc.

***ESBL* Producer Variants**

*ESBL* producer strains were noticed only from Abu-Ghraib region, in which they detected from seven (7.77%) samples: six (6.66%) strains from soft cheese and one (1.11%) from its whey. Those variants noticed during calculating and subtracting the differences in millimeter between diameters of Cefpodoxime/clavulanic acid (CD01) 10/1µg (must be the larger zone) & Cefpodoxime (CPD10) 10µg(must be the smaller zone) zone of inhibition, so that any result larger than 5 millimeter indicate phenotypically *ESBL* production as in photograph (2):

Photograph (2): *ESBL*producer variants isolated from soft cheese and its whey from Abu-Ghraib region in Iraq, and detected in Cefpodoxime combination disk on Muller-Hinton agar. Double inhibition technique may reveal phenotypically the presence of extra resistance enzymatic series of *ESBL* producers especially in isolates from Abu-Ghraib region. This behavioral extra resistance strategy might be linked to biofilm secretion and capsule. This resistance strategy confirmed by routine antibiotic susceptibility pattern for selected antimicrobial agents especially for CTX resistance isolates as noticed in table (4). In general, this risk predicted from accurate species identification. Metallo-carbapenemases were slowly emerging and present a new detection challenge, partially because carbapenem resistance does not always accompany enzyme production, as well as *KPC* lineage may phenotypically different from *NDM-1.ESBLs* are of increasing concern as mutants of classical *TEM* and *SHV* plasmid-mediated. They are prevalent in *K. pneumoniae* as nosocomial strains, but we noticed them in food (soft cheese and its whey) as an indicator of case history of isolates, community, and livestock epidemiological behavior of frequency and distribution pattern. *ESBL*-mediated resistance to cephalosporins is not always obvious in disc or dilution tests as the *MICs* of cephalosporins for producers can be as low as 0.5 mg/L and the inhibition zones of discs are correspondingly large (Livermore and Brown, 2001), so that Oxoid Cefpodoxime Combination Kit (Oxoid – Remel, 2016) might aid in phenotypic detection of *KPC* strains.

**Conclusion and Recommendation**

Data showed contamination of raw white soft cheese and its whey made from Cows raw milk in Baghdad with biofilm

producing and multidrug resistant strains of *K. pneumoniae* with noticed *ESBL* phenotypes, thus we recommend monitoring of soft cheese production cycles, handlers, cheese makers, soft cheese containers, utensils, equipment, environment, vectors, transportation and storage stages through application of sanitation practices during cheese processing to overcome or reduce these public health problems, aswell as application of up to date diagnostic tools for reducing dangerous foci of *K. pneumoniae* and their transmission as soon as possible with treating of diseased cases carefully, monitoring active and passive carriers, and designing future hazard analysis critical control points strategies to controlling the epidemiological distribution and frequency pattern of *K. pneumoniae*in regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya.

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