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Detection of *Klebsiella pneumonia* in raw food and their antibiotic resistance

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ABSTRACT

Twenty two bacterial isolates (A01-A22) were isolated from juices, fruits and vegetables salad in restaurants at Taif City-Kingdom of Saudi Arabia, then characterized by morphology, biochemical reactions and confirmed by API 20 E system. Furthermore, all isolates were identified by partial 16SrRNA gene sequencing. All isolates were identified by 95-99% identity, except strain A13 was not complete. Eleven isolates out from twenty two were identified as *K. pneumnonia*, nine were *K. quasipneumnonia*, and only one named by K. oxytoc. In addition, antibiotic resistance profiles of isolated strains were determined. The highest resistance rate was scored against Penicillin and Erythromycin by 100 %. Multidrug resistance pattern of the sixteen strains was observed against 3 types of antibiotics were used. In conclusion, the high rate of antibiotic resistance in bacterial strains may be incidence from manner was used in agriculture. This result may be caused major implications for human and animal health with adverse economic implications. Results showed the high health risk associated with consumption of raw vegetables and could play public health hazard. It is recommended that seller and food handlers within the respective restaurants should make conscious efforts to decontaminate and properly handle the vegetables prior to its salad preparation.

KEYWORDS: Klebsiella pneumonia ,antibiotics resistance

INTERODUCTION

Klebsiella pneumonia are rod shape enteric bacteria, gram negative, nonmotile, encapsulated, facultative anaerobic, and lactose fermenting. They are the most important members in family of *Enterobacteriaceae*. These bacteria are widely dispersed in intestines of humans, urinary tract and found naturally in the soil, water, raw vegetables or fruits [24,45,71]. *K. pneumonia* was isolated for the first time in 1882 by Friedlander from the lungs of patient. This encapsulated bacterium, initially named Friedlander's bacillus. Later this organism was given the generic name *Klebsiella spp.* in 1886, and it was known ubiquitously in the worldwide [28]. Strains of *Klebsiella spp.* were linked to wide variety of diseases in humans. This bacterium has grown to be important pathogens in nosocomial infections in United States [49]. It was described as a saprophyte microorganism not only colonizing the human gastrointestinal tract, skin and nasopharynx, but also able to cause osteomyelitis, urinary and biliary tract infections. *Klebsiella pneumonia* was recorded as a first case of infection in Houston, texas, the patient suffered from symptoms of gastroenteritis rapidly lead to multiorgan failure [63]. Recently, *K. pneumonia* were secluded from spider herbs and dried bush okra In African [51]. Another report from Libya showed that *K. pneumonia* was existing in fruit juices [27]. *K. pneumonia* were recognized as an important pathogens in raw food and caused food illness [30].

There are many sources of entry of microorganisms into fruit juices. The major of these source were by environmental exposure, improperly washing, used of un hygienic water for dilution, dressing with ice, prolonged preservation without refrigeration. Fresh juices and salad were equipped to consumption, quick methods of cleaning utensils, miss handling and extraction, they could often provide evidence to be a public health threat [42, 12].

Fresh vegetables and fruits are well standard for their rich nutritive value, mineral, vitamin content, and often consumed raw or minimally processed. They were the most common or one of the principal outbreaks of food borne disease in the worldwide. Fresh food can become polluted throughout the food chain from farm to table, via, for example, used unprocessed water for fertilization, irrigation or pest control, contact with animal or human excreta, food handlers or by cross contamination in domestic environments [16,74]. Vegetables and fruits were documented as source of contamination with *K. pneumonia* [61].

K. pneumonia were dispersed from fresh vegetables, whereas collected from cultivated lands, supermarket, and ready-to-eat vegetable salads [21]. *Klebsiella spp*. were the dominating species in fruits, and vegetables [41]. In Kumasi, *K. pneumoniae* (18%), and *E. coli* (2.2%) were isolated from street vending food such as ready-to-eat red pepper salad and macaroni [22]. In Malaysia hypermarkets, *K. pneumoniae* was to be considerably more frequent (100%) and (82.5%) in lettuce and cucumbers, respectively. *K. pneumoniae* contamination was lowest in carrot samples (30%) [61]. non hemolytic bacteria were identified from vegetable salads sold in restaurants located at Okada town; *Staphylococcus epidermidis, E.coli, and K. pneumonia* [58]. *Staphylococcus aureus* (29.2%) was the most frequently isolated followed by *Klebsiella spp* (12.5%) from raw vegetable [19].

Microbial hazards continue to be one of the biggest threats of food safety [5, 18]. Recently, the highest Food borne illness was associated with the eating of raw fruits, vegetables, juices and salad. Each year, millions of individuals become ill from food disease, and those salad can be sources of pathogens transmission [60,75]. More than 90 percent of the personal belongings of food poisoning each year caused by; *E.coli, Staphylococcus aureus, Listeria monocytogenes and K. pneumonia* [67].

In the USA, centers for disease control and prevention (CDC) reported that plant caused about 46 % of internally acquired food –borne illnesses from 1998 to 2008 [44]. The majority of these plant based food borne illness were coupled with edible horticultural crop; that often consumed without any heat or chemical treatment to kill pathogens. While the obesity epidemic in Europe and in North America has promoted consumers to eat healthier diets which include more fresh vegetable to avoid chronic health problems (31, 59). Finally, current guidelines and regulations to minimize risks connected to wild animal activity in the production environment [35].

World Health Organization has five keys as a tool for food safety education in the African Region. The keys to safer food were: Keep clean; separate raw and cooked; cook thoroughly; keep food at safe temperatures; and use safe water and raw materials [52]. Furthermore, the food safety applies and practices of street vendors were evaluated in Gizan city Saudi Arabia [50]. They were identified *E. coli, Staphylococcus, Bacillus and Salmonella spp* from different types of street food.The saving of food hygiene was difficult to practice at street in setting, where personal hygiene knowledge these people decided that; hand washing was necessary (52%) and bathing regularly (74%).

In recent years, the occurrence of antibiotic resistant strains of a number of pathogenic bacteria as well as *E.coli, Salmonella spp., Klebsiella spp.* has emerged as another health alarm all over the world [29]. Antibiotics are often used against disease caused by *Klebsiella spp*. but these pathogens are becoming progressively more antibiotic resistant, so that many are now labeled as MultiDrug Resistant (MDR) [40-4]. These strains causing complicated food borne illness varies significantly between regions and countries [53].

The presence of antibiotic resistances both in normal flora and pathogenic microorganisms in fresh vegetables may contribute to horizontal distribution of resistances between different isolates, genera and species. The occurrence of resistance genes on transferable elements facilitates, which dispersed of resistance and the wide spread use of antibiotics allows direct selection of resistances [33]. Commercial animal husbandry was prime areas of antibiotic resistance development. The use of large amount of antibiotics in plant agriculture could lead to a selection of resistant bacteria; used the contamination water for irrigation and transfer the manure of animal farm to agriculture field could spread resistance bacteria to plant. Finally, the incidence of antibiotic-resistant bacteria in fresh vegetables constitutes an extra concern for consumer safety.

The survival of coliform bacteria as well as their antibiotic susceptibilities in fresh vegetables, as an indicator of their microbiological quality and their potential as a risk factor for consumer's health [21]. They were identified isolates species belonging to *Klebsiella, Enterobacter*, and other genera. Most isolates were resistant to ampicillin, and to amoxicillin/clavulanic acid; even if resistances to other chemotherapeutic agents were rare, some isolates showed multi resistance to 3-5 agents [36]. The aims of this study are to isolate and identify *Klebsiella spp.* existing in raw food such as vegetables, fruits and juices at Taif restaurants and study their antibiotics resistance.

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MATERIALS AND METHODS

2.1. Samples collection:

Samples were obtained from different sources such as fruits (Kiwi, Orange, Guava, Green apple, Red apple, Mango, Banana, Strawberry, Melon, Pear, Pineapple, Avocado, Fruit salad, Grape, from vegetables salads (Tabbouleh, Corn, Cabbage, Russian, Fattoush, Hot vegetable, Greek, Vegetables and cheese, Caesar chicken, Milk and cucumber, Metabel, Chickpeas). Eighty samples were collected from Taif City Region and used for isolation *K. pneumonia* belong to family of Enterobacteriaceae. All samples were immediately transferred under aseptic condition in ice box to the laboratory.

2.2. Isolation of bacterial strains:

2.2.1. Vegetables and fruits samples:

Vegetables and fruits samples 1gm were soaked in 9ml of normal saline solution (8.5gNacl / L) homogenized for 2min, suspensions were serially diluted up to 10^6. One ml each dilution was transferred to sterile petri dishes and then about 15 ml of Nutrient and MacConky agar were poured. All plates were converted and incubated aerobically at 37^{o}C for 24 h. After incubation, counted the total numbers of coliform and aerobic bacteria for each collected sample. Individual, colonies on MacConky agar plates were selected according to their morphological differences such as color, shape and size then transferred into 10 ml sterile MacConky broth. The inoculated tubes were incubated aerobically at 37 ^{o}C for 24-48 hrs. A lactose fermenting colonies were spread on nutrient agar plates. According to the standard method of the microbiological examination, streak plate technique was applied in the present study to isolate and purify culture bacterial strains [34] in nutrient agar plates. For short- term preservation at 4 ^{o}C, pure single colonies were streaked on nutrient agar tubes (slant). All isolates were periodically sub-cultured every two months on specific medium. Standard biochemical tests were performed following standard procedures [14].

2.3. Preliminary identification of isolates:

All isolates were confirmed to the genus level by colony and cell morphology, Gram stain, motility, oxidase, urease, triple sugar iron, methyl red, indole, vogus proskaure, citrate, [15]. Furthermore, All isolates were confirmed by using the API 20E [37].

2.3.1. Identification of isolates by using API:

All isolates were confirmed by using API 20E strips (BioM´erieux), following the manufacturer's instructions. This Analytical profile index (API) is a plastic strip holding twenty mini-test tubes used in identification of Enterobacteriaceae. It is a ready- to-use, microtube system designed for the performance of some standard biochemical tests from isolated colonies of bacteria on plating medium.

The positive reactions were compared with the differentiation chart provided with the Analytical profile Index (API) to determine the genus and/or species of the organism [48].

2.4. Partial sequencing of 16S rRNA gene:

2.4.1. DNA extraction from culturable bacteria:

The genomic DNA of culturable bacterial isolates was extracted using QIAamp DNMini kit (Qiagene) according to manufacture,s protocol.

2.4.2. DNA sequencing:

The PCR –amplified 16S rDNA fragments were amplified using two universal primers; fD1 (5' agagtttgatcctggctcag 3') and rP2 (5' acggctaccttgttacgactt 3') [73]. The reaction mix was composed of × µL Template DNA, 2 µL BigDye-Mix, 1 µL universal primer (10 µmol), 1µL Taq DNA polymerase, and HPLC water to a final volume of 10 µL. The amount of template DNA applied was dependent on the concentration of target sequences to obtain about 10 ng DNA in the final mix. The PCR program was as follows ; initial denaturation at 96^{o}C for 2 min (1 cycle), denaturation at 96^{o}C for 10 s (30 cycle), annealing at 45 ^{o}C for 5 s (30 cycle),extension at 60 ^{o}C for 4 min (30 cycle), and then cooling at 4 ^{o}C. The PCR product was purified using an DNA-purification kits as recommended by the manufacturer and then sequenced. PCR fragments were analyzed by cycle sequencing, using the BigDye terminator cycle sequencing kit (Applied Biosystems, U.K.). This sequence step was commercially carried out by Macrogen Inc.,Seoul, South Korea, through 16S rDNA sequencing using universal primer, 518F (5' ccagcagccgcggtaatacg 3')[3]. The obtained partial nucleotid sequences of the Materials & Methods **35**16S rRNA gene, were aligned using Clustal W from MEGA 4.0 software [70] and compared with the homologous sequences of the type strains, available in the GenBank database.

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2.5. Antibiotic Susceptibility Test:

Antimicrobial susceptibility testing was done on Mueller-Hinton agar (MHA) (Merck biolab, Gauteng) by the standard disc diffusion method recommended by the Clinical and Laboratory Standards Institute [13]. The investigation of the antibacterial activity of eight types of antibiotics, synthesis nano silver (chemically, purchased from Egypt) were performed against 32 isolates. Active isolates (about 22 h old) were transferred into test tubes containing 5 mL sterile normal saline. The turbidity of the suspension was adjusted to 0.5 McFarland standards (equivalent to 1.5×10^8 CFU/100 mL). Sterile swabs were soaked into the bacterial suspensions and used to inoculate the MH agar plates by spreading uniformly on the surface of the agar. Antibiotic discs were placed equidistant from each other on the agar surface. After incubation for 24 hrs. at 37°C, reading was done. The effect of antibiotics synthesis and nano silver against isolate were estimated by the appearance of clear zones around the discs. The diameter of the halo of growth inhibition was measured and expressed in mm.

RESULTS AND DISCUSSION

3.1. Isolation of bacteria from raw food:

Twenty-nine types of raw food were used in the present study as source for isolation some types of *Klebsiella* spp. belong to family Enterobacteriaceae. All samples (70) were collected from local restaurant in Taif City, Saudi Arabia. These samples were divided into three types; juice, fruits and vegetables salad. Thirty-three samples were collected from vegetables salad such as; vegetables Tabbouleh (5),Corn(1), Cabbage(4), Russian(3), Fattoush (1), Hot vegetable(5),Greek(1),Vegetables (3), Caesar (1),cucumber(2), Metabel(3), Chickpeas(4). Thirty-four samples were collected from juices such as; Kiwi(2), Orange(2), Guava(3), apple(4), Mango (1), Banana(3), Strawberry (2), Melon(4), Pear(1), Pineapple(2), Avocado (3), Grape(1),Cocktail (5), Pomegranate(1), three samples were collected from fruits salad. The data present in Table (1) showed results of total counts of aerobic bacteria and coliform among from juices, fruits, vegetables salad samples.

All the collected fruits and vegetables samples in this study were scored highly variability in bacterial counts. Even on samples with particularly high number of bacteria, no visible sign of defect was observed on them. Overall, highest count from aerobic and coliform bacteria were found in salad e.g., Tabbouleh, Vegetable, Caseer, Greek, Chickpeas, fruit and juices e.g. Avocado, Melon, Cocktaill. kiwi juice was not detected any count from coliform bacteria, while, it scored high amounts from aerobic bacteria.

High presence of coliform organisms in salad, these results revealed the possibility of spreading enteric diseases to the consumers. Similar results were also reported by other authers[38,21,54]. Higher level of total coliform bacteria was showed in Tabbouleh and Caesar salad respectively, it is presented in this study, the same results concluded by [39,62,58].

The mean aerobic bacterial of vegetable, fruits salad obtained in this study were similar with the recent study conducted by Food and Drug administrations. This study showed that the total bacterial load ranged from 4 to 8.3 log [9]. The same data reported in singapore [64]. While, the maximum microbial load in Mutabbel salad were scored Riyadh City Kingdom of Saudia Arabia [39].

A potential sources of the contamination may be due to water used in rising and processing, untreated manure used in soil of the fruits and vegetables prior to sale. European Commission stated that cutters and slicers used in preparation salad dressings, improper handling and producing conditions can be effective sources of contamination [20, 7,1]. Finally, the contamination of juices was mainly due to broke quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils, contaminated ice, poor personal and demotic hygiene, peeling fruits beforehand [69].

Table 1: Results of aerobic and coliform bacteria counts in the collected samples (juices, fruits, vegetables).

Name of sample	(NO.)	Viable cell count CFU / g 10^4 aerobic bacteria	CFU / g 10^2 coliform count
Tabbouleh	5	122.4	193
Corn	1	0.70	1.80
Cabbage	4	31.7	134
Russian	3	1.88	21.0
Fattoush	1	1.20	22.7
Hot vegetable	5	12.2	31
Greek	1	124	179
Vegetables	3	128	172
Caesar	1	134	177
Cucumber	2	3.40	10.4
Metabel	3	0.30	6.20
Chickpeas	4	139	183
Kiwi	2	200	ND
Orange	2	4.40	77.2
Guava	3	16.3	95.0

Apple	4	3.13	1.60
Mango	1	0.10	1.90
Banana	3	3.57	9.48
Strawberry	2	1.21	11.3
Melon	4	124.9	195
Pear	1	2.26	12.9
Pineapple	2	2.26	4.30
Avocado	3	162	130
Grape	1	0.35	87.5
Cocktail	5	126	106
Pomegranate	1	0.11	1.0
Fruit salad	3	119	115

CFU: Cell Forming Units; ND not detected

3.2. Preliminary identification:

Twenty-two isolates showed differences in their morphological and biochemical characters were selected for further work. These isolates named from A1 to A22 and subjected for further molecular identification. A summary of the twenty two isolates that satisfied both the preliminary and confirmatory identification characteristics for Enterobacteriacea are shown in Table (2). All isolates were fermented lactose, non motile, Gram negative (*G- Ve*) short rods shape. These isolates were fermented the sugars in the TSI and produced gas from the fermentation of sugars. All isolates have the ability to hydrolyze citrate, VP and urease test was positive. The same steps of study and identify isolates were carried out by authers [32]. The API 20E test results indicated that the bacteria isolates belonging to *Klebsiella pneuomniae* were identified Morphological examination revealed that their colonies were large, circular, convex, grayish white and mucoid on nutrient agar. While on MacConkey' s agar lactose fermenting (pink) colonies were detected. All isolates were identified as *Klebsiella pneumonia.* These results either by the biochemical reactions or with API system were confirmed that all isolates are named *Klebsiella pneumonia* and the agreement with the results conducted by other authors. [41,66,62].Fig.1.Illustrated representative light microscope images for morphology of the isolated bacteria.

Table 2: Source of isolation (Raw food), isolate numbers and some morphological and biochemical characters of the purified Straight Rods

Source of isolation (Fresh food) (NO.)	Isolate NO.	Gram stain	Motality	oxidase	Methy l red	Citrate	Trible Sugar Iron	Urease	indol	Voges proskauer
Vegetables and cheese salad	A01	-	-	-	-	+	+	+	+	+
Metabel	A02	-	-	-	-	+	+	+	+	+
Fruit salad	A03	-	-	-	-	+	+	+	-	+
Chickpeas	A04	-	-	-	-	+	+	+	-	+
Metabel	A05	-	-	-	-	+	+	+	-	+
Metabel	A06	-	-	-	-	+	+	+	+	+
Metabel	A07	-	-	-	-	+	+	+	+	+
Russian salad	A08	-	-	-	-	+	+	+	-	+
Chickpeas	A09	-	-	-	-	+	+	+	-	+
Chickpeas	A10	-	-	-	-	+	+	+	-	+
Hot vegetable salad	A11	-	-	-	-	+	+	+	-	+
Hot vegetable salad	A12	-	-	-	-	+	+	+	+	+
Russian salad	A13	-	-	-	-	+	+	+	-	+
Hot vegetable salad	A14	-	-	-	-	+	+	+	-	+
Hot vegetable salad	A15	-	-	-	-	+	+	+	-	+
Metabel	A16	-	-	-	-	+	+	+	-	+
Chickpeas	A17	-	-	-	-	+	+	+	-	+
Guava juice	A18	-	-	-	-	+	+	+	-	+
Fattoush	A19	-	-	-	-	+	+	+	-	+
Fruit salad	A20	-	-	-	-	+	+	+	-	+
Corn salad	A21	-	-	-	-	+	+	+	-	+
Pineapple juice	A22	-	-	-	-	+	+	+	-	+

3.2.1.Identification by API system:

The results obtained from API 20E represented in Fig. (1) indicated that, the isolates under study were belong to *Klebsiella pneumonia.*

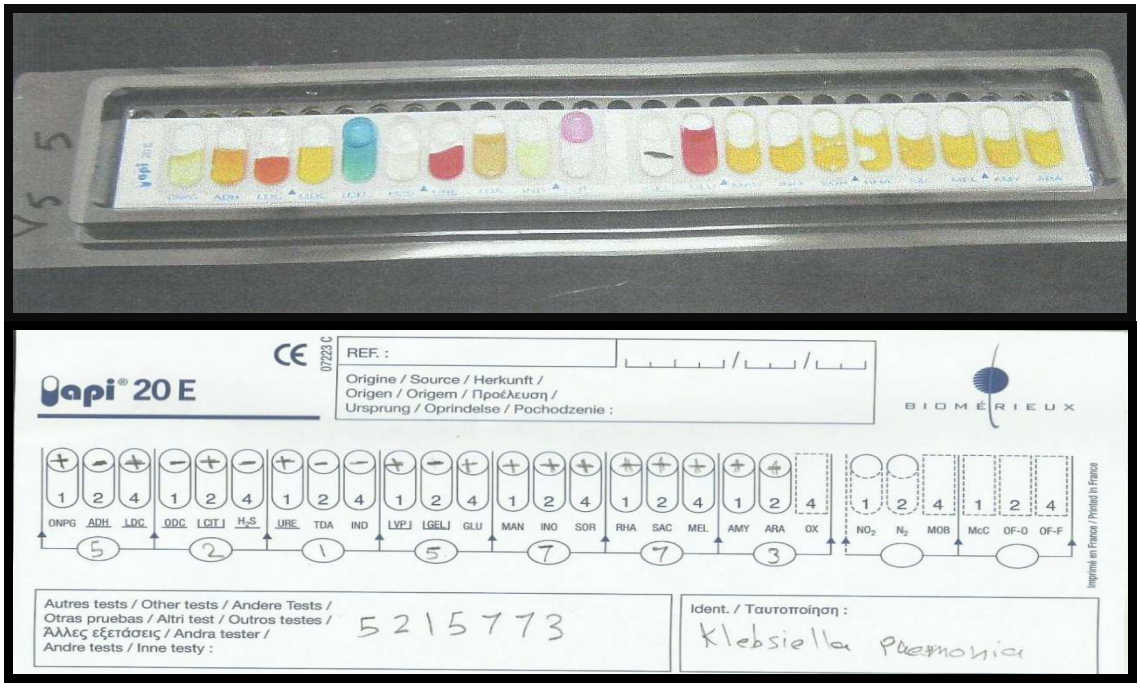

Fig. 2: Identification of isolates by API 20 E system.

3.3. Molecular Identification:

Twenty tow strains (A1-A22) were identified by determine and analysis of the partial sequence of 16S rRNA gene, except isolate NO.13 the sequence was not complete to identify. The species were initially determined by the BLAST program on NCBI (http://www.ncbi.nim.nih.gov/) based on the 16S rRNA sequences of type strains. The identity and coverage percentage of isolates were presented in Table 3. All isolates were identified with type stain of *Klebsilla spp.*, and the percentage of identity were ranged 95-99%, except strain A13. Eleven isolates out from twenty two were identified as *K. pneumnonia*, nine were *K. quasipneumnonia,* and only one named by K. oxytoc. In this study the sources of obtained isolates were differ from vegetable, fruit salad and juices Table 4. The most of isolates were collected from metable, vegetable, and chickpea salad. The prevalence of *Klebsilla spp.*in vegetable samples were found to be 22.8%.The same results was conducted by authors, they are determined *Klebsilla spp*.in vegetable samples to be 33.3 and 65% respectively [41,61]. Moreover, *K. pneumoniae* was survival to be very high in lettuce (100%) and cucumber samples (82.5%) and lowest in carrot samples (30%) [23], While, other research counted *Klebsiella spp* only in tomato samples [62] and from the vegetable salad [58]. On the other hand, the highest numbers from *E.coli* in all salad samples (vegetable, metabble, tabouleh, hummus and fattoush) [39].

Leafy vegetables (such as lettuce, celery, spinach, basil, leek, Chinese cabbage and parsley) were given that a large surface areas and topographical features which can encourage the attachment of microorganisms. Besides these vegetables have high relative humidity which favors the spread and continued existence of bacteria on plant surfaces as mentioned [2]. The dominance of *Klebsilla spp*. amongst the bacterial genera identified from the vegetable salad is not surprising by authors. They reported that the majority of bacteria found on the surface of plants are usually gram negative bacteria belong to Enterobacteriaceae [46].

These results are in agreement with a previous report by author, who reported that food materials of plant and animal origins either cooked and uncooked are predominately contaminated by coliforms, which are mainly non hemolytic [17]. The exposing vegetables to various types of cutting has been shown to increase result to seven fold increase microbial numbers [26].

The present study recorded that the prevalence of *K. pneumonia* was in 18.2% in juices and mixed fruits samples. These results are agreement with authors, they reported that *Klebsiella* spp was spoilage vegetables and fruits [63], and spoiled apple juice [11], also spoilage frozen vegetables [47]. Similar finding, stated that the percentage of *Klebsilla spp.* in vendor juice were 3% [69], while prevalence of *Klebsilla spp.* was found to be 31.2% [41].While Eni *et al*., (2010) *Klebsiella spp.* were secluded from vegetables but not found in all fruit samples [19]. Other sources of contamination of vegetables and fruits, including soil, organic fertilizers and irrigation water [2,6,10,43].

Table 3: Identity and coverage percentage according to the obtained 16S rRNA sequence.

Isolate code No.	Submission in GenBank		Most related bacteria in Genbank		Query covering %	Identities (%)
	Accession No.	Released names	Accession No.	Names of bacteria		
A01	KU711902	*Klebsiella* sp. A1-KS1 16S ribosomal RNA gene, partial sequence	NR_112010.1	*Klebsiella oxytoca* strain JCM1665 16S ribosomal RNA gene, partial sequence	97	95
A02	KU711918	*Klebsiella pneumoniae* strain A02 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	99	96
A03	KU711903	*Klebsiella* sp. A3-KP2 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	98	96
A04	KU711904	*Klebsiella sp*. A4-KS2 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence*	95	96
A05	KU711905	*Klebsiella sp*. A5-KP3 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	97	96
A06	KU711906	*Klebsiella sp*. A6-KS3 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	98	96
A07	KU711907	*Klebsiella sp*. A7-KS4 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	97	96
A08	KU711908	*Klebsiella sp*. A8-KP4 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	96	95
A09	KU711909	*Klebsiella sp*. A9-kP5 16S ribosomal RNA gene, partial sequence	NR_074913.1	*Klebsiella pneumoniae* subsp. pneumoniae MGH 78578 strain ATCC 700721; MGH 78578 16S ribosomal RNA	96	96
A10	KU711910	*Klebsiella sp*. A10-KP6 16S ribosomal RNA gene, partial sequence	NR_074913.1	*Klebsiella pneumoniae* subsp. pneumoniae MGH 78578 strain ATCC 700721; MGH 78578 16S ribosomal RNA, complete sequence	94	96
A11	KU711919	*Klebsiella pneumoniae* strain A11 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence*	99	97
A12	KU711911	*Klebsiella sp*. A12-KP8 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	95	96
A14	KU711912	*Klebsiella sp*. A14-KP10 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	97	95
A15	KU711913	*Klebsiella sp*. A15-KP11 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	97	96
A16	KU711914	*Klebsiella sp*. A16-KP12 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	99	99
A17	KU711920	*Klebsiella pneumoniae* strain A17 16S ribosomal RNA gene, partial sequence	NR_134063.1	*Klebsiella quasipneumoniae* subsp. similipneumoniae strain 07A044 16S ribosomal RNA, partial sequence	100	96
A18	KU711915	*Klebsiella sp*. A18-KP14 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	95	95
A19	KU71	*Klebsiella pneumoniae* strain	NR_1176	*Klebsiella pneumoniae* strain DSM 30104	100	99

	1921	A19 16S ribosomal RNA gene, partial sequence	83.1	16S ribosomal RNA gene, partial sequence		
A20	KU711916	*Klebsiella sp*. A20-KP16 16S ribosomal RNA gene, partial sequence	NR_114506.1	*Klebsiella pneumoniae* strain ATCC 13883 16S ribosomal RNA gene, partial sequence	97	96
A21	KU711922	*Klebsiella pneumoniae* strain A21 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	100	99
A22	KU711917	*Klebsiella sp*. A22-KP18 16S ribosomal RNA gene, partial sequence	NR_117683.1	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	98	96

Table 4: Sources of isolation and suggested name of bacterial strains given according to the partial sequencing of 16S rRNA gene.

Strain name given after partial of 16S rRNA sequencing		Sources of isolation (No)	
Klebsiella oxytoca	A01	Vegetables	(1)
Klebsiella pneumoniae	A02	Metabel	(3)
Klebsiella pneumoniae	A03	Fruit salad	(3)
Klebsiella quasipneumoniae	A04	Chickpeas	(4)
Klebsiella quasipneumoniae	A05	Metabel	(3)
Klebsiella quasipneumoniae	A06	Metabel	(3)
Klebsiella quasipneumoniae	A07	Metabel	(3)
Klebsiella pneumoniae	A08	Russian salad	(3)
Klebsiella pneumoniae	A09	Chickpeas	(4)
Klebsiella pneumoniae	A10	Chickpeas	(4)
Klebsiella quasipneumoniae	A11	Hot vegetable salad	(5)
Klebsiella quasipneumoniae	A12	Hot vegetable salad	(5)
Klebsiella quasipneumoniae	A14	Hot vegetable salad	(5)
Klebsiella quasipneumoniae	A15	Hot vegetable salad	(5)
Klebsiella pneumoniae	A16	Metabel	(3)
Klebsiella quasipneumoniae	A17	Chickpeas	(4)
Klebsiella pneumoniae	A18	Guava juice	(2)
Klebsiella pneumoniae	A19	Fattoush	(1)
Klebsiella pneumoniae	A20	Fruit salad	(3)
Klebsiella pneumoniae	A21	Corn salad	(1)
Klebsiella pneumoniae	A22	Pineapple juice	(2)

3.4. Antibiotic resistance profile:

The present study showed the effect of eight commonly antibiotics against all isolated strains Table 5. All strains were resistance to Penicillin G and Erythromycin by 100%. Sixteen out from 22 displayed multidrug resistances against 3 types of antibiotics used in this study. The *Klebsiella pneumonia* A2 was sensitive for the seven tested antibiotics. Most of *Klebsiella pneumonia* isolates were displayed highly sensitive to Ciprofloxacin, Ceftazidime, Oxytertracycilne (100%). These results are agreement with the finding by authors [65]. They concluded that seventy and sixty –nine percentage of *Klebsiella pneumonia* were sensitive to Ciprofloxacin, and Ceftazidime.

Table 5: Effect of Antibiotics resistance profile index of the bacterial isolated from raw food.

Strains		**E15**	**P10**	**N A30**	**C IP5**	**C AZ30**	**OT30**	**A MP10**	**CN 10**	**N O. of antibiotic resistance**
Klebsiella oxytoca	A01	I	R	-	-	-	-	R	-	2
Klebsiella pneumoniae	A02	I	R	-	-	-	-	-	-	1
Klebsiella pneumoniae	A03	I	R	-	-	-	-	R	-	2
Klebsiella quasipneumoniae	A04	R	R	-	-	-	-	R	-	3
Klebsiella quasipneumoniae	A05	I	R	-	-	-	-	R	-	2
Klebsiella quasipneumoniae	A06	I	R	-	-	-	-	R	-	2
Klebsiella quasipneumoniae	A07	R	R	-	-	-	-	R	-	3
Klebsiella pneumoniae	A08	R	R	-	-	-	-	R	I	3
Klebsiella pneumoniae	A09	R	R	I	-	-	-	R	-	3
Klebsiella pneumoniae	A10	R	R	-	-	-	-	R	-	3
Klebsiella quasipneumoniae	A11	R	R	-	-	-	-	R	-	3
Klebsiella quasipneumoniae	A12	R	R	-	-	-	-	R	-	3
Klebsiella pneumoniae	A13	R	R	-	-	-	-	R	-	3
Klebsiella quasipneumoniae	A14	R	R	-	-	-	-	R	-	3
Klebsiella quasipneumoniae	A15	R	R	-	-	-	-	R	-	3

Klebsiella pneumoniae	A16	R	R	-	-	-	-	R	-	3
Klebsiella quasipneumoniae	A17	R	R	-	-	-	-	R	-	3
Klebsiella pneumoniae	A18	I	R	-	-	-	-	R	I	2
Klebsiella pneumoniae	A19	R	R	-	-	-	-	R	-	3
Klebsiella pneumoniae	A20	R	R	-	-	-	-	R	I	3
Klebsiella pneumoniae	A21	R	R	-	-	-	-	R	-	3
Klebsiella pneumoniae	A22	R	R	-	-	-	-	R	-	3
Antibiotic resistance rate %		100	100	5	0	0	0	95	14	

Inhibition zone diameter were measured inclusive of the diameter of the discs(-) sensitive ; (I) Intermediate ; (R) Resistant according to the table 2 (Oxoid Manual.,1982). E (Erythromycin),P (Penicillin G),NA (Nalidixic acid), CIP (Ciprofloxacin), CAZ (Ceftazidime), OT (Oxytertracycilne), AMP(Ampicillin),CN(Gentamicin). (Antibiotic resistance rate =(number of strains resists certain antibiotic\ total number of tested strains) x 100.

Remarkably, *Klebsiella spp.* strains showed resistance to ampicillin by 95.65%. Same finding has been previously demonstrated by authors [55,56]. They reported that *Klebsiella spp* were resistance to ampicillin by 100 %. The researcher proved that *Klebsiel la pneumonia* was isolated from fresh vegetables and showed a high resistance to ampicillin [21].

In general, the highest antibiotic resistance rate (100%) was recorded by the isolated strains was for Penicillin, Erythromycin, and then followed by ampicillin (95%). The other antibiotic resistance rate was ranged from 0 to 14%. In agreement with this results conducted by authors [8]. They reported that the most isolates from *Klebsiella pneumonia* contaminated raw vegetables were multidrug resistant.

Statistical data and evidences from researches prove that multi drug resistant bacteria are emerging worldwide which causes many public health troubles and challenges to healthcare. Antimicrobial resistance is a global concern not only because it kills but because it increases health costs and threatens patient care [68]. Moreover, uses of broad spectrum antibiotics, insufficient aseptic condition and technique with insufficient control of infections spread had aggravated this problem.

Therefore, there is need for concerted efforts between Clinicians and Public health workers in educating the people from this condition on the menace of indiscriminate use of antibiotics, especially when not arranged by Physicians. Government, through appropriate agencies should describe out drug sales and use policies/legislatures that will put to check the sales of antibiotics to people without a supporting manuscript for prescription by qualified personnel [57].This study indicated that minimally processed raw food (juices, vegetables and fruits salad) has potential health risk due to the isolation of *Klebsiella spp.* and associated this pathogen due to lack of strict hygiene control measures in the food chain. Therefore, more concern needs to be taken during preparation; use of treated waste water for irrigation, improper cleaning of storage and preparation areas and unclean utensils, workers should be educated about the path of contamination and observed them during handling and marketing salad or juice.

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