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Isolation, Characterization and Antimicrobial Resistance Patterns of Lactose-Fermenter Enterobacteriaceae Isolates from Clinical and Environmental Samples

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Abstract

The lactose-fermenter Enterobacteriaceae are the most frequent cause of clinical infection in our country. The objective of this study was to isolate and identify the most common lactose-fermenter Enterobacteriaceae from clinical samples, including urine, blood, wounds, and sputum, obtained from the local hospital and from environmental samples from a chicken farm, agriculture soil, and water from the Tigris River in Baghdad City. The study also aimed at establishing the antibiotic resistance patterns of the isolated bacteria. A total of 155 bacterial isolates were identified from 10 genera according to the Vitek 2 system. The most common bacterial isolates from the clinical and environmental samples were *Escherichia coli* and *Klebsiella pneumoniae*, respectively. The antibiotic resistance patterns showed that all clinical and environmental isolates were multidrug resistant to *β*-lactam (except carbapenems) drug and aminoglycosides and more sensitive to carbapenems.

Keywords

Enterobacteriaceae, Lactose Fermenters, Clinical, Environmental Samples

1. Introduction

Enterobacteriaceae are distributed worldwide. They are found in soil, water, fruits, meats, eggs, vegetables,

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grains, flowering plants and trees, and in animals from insects to man [1]. Enterobacteriaceae are Gram-negative rods with a length of 1 - 3 µm. They are facultative anaerobes, oxidase-negative, catalase positive, and grow on MacConkey agar, and their natural hosts are human and animal intestines [2]. There are 44 genera and approximately 176 species [1]. The normal flora include *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Morganella* spp., *Proteus* spp., *Providencia* spp., and *Serratia* spp., and the obligate human pathogens include *Salmonella* spp., *Shigella* spp. and *Yersinia* spp. [3]. Enterobacteriaceae may account for 80% of clinically significant isolates of Gram-negative bacilli and 50% of clinically significant bacteria in clinical microbiology [4]. Some Enterobacteriaceae species have only been identified based on their16S rDNA sequence and have not been isolated or characterized biochemically [1].

The Enterobacteriaceae family is divided into three groups on the basis of lactose fermenter as follows: I-lactose fermenters, such as *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp.; II-late lactose fermenters, such as *Citrobacter* spp. and *Serratia* spp.; and III-lactose nonfermenter, such as *Edwardsiellatarda*, *Hafnia*, *Morganella morganii*, *Proteus* spp., *Providencia* spp., *Salmonella* spp., *Shigella* spp., and *Yersinia* spp. [2] [5]. Common water microbiological indicators are used as markers of risk to human health, including total coliform, *Escherichia coli*, enterococci, *Clostridium perfringens*, and *Pseudomonas aeruginosa* [6] [7]. Vegetable contamination can arise due to the treatment of soil with organic fertilizers, such as sewage sludge and irrigation water, as well as from the ability of pathogens to persist and proliferate in vegetables [8] [9]. *E. coli* is one of the common normal floras (N.F.) of the gastrointestinal tract of poultry and humans. It is also present in other animals but some strains that pathogenic to both poultry and humans [3] [10].

Pathogenic Gram-negative bacteria are treated with antibiotics, and the efficient agents are fluoroquinolones, beta-lactams and aminoglycosides [11]. Resistant strains from the poultry gut readily contaminate poultry carcasses, and when consumed, they alter or affect the endogenous normal flora of humans [12]. Gene transfer occurs primarily *in vivo* between gastrointestinal tract bacteria (N.F.) and pathogenic bacteria because identical resistant genes are present in diverse bacterial species from different hosts [13]. The objective of this study was to determine the incidence isolates and antimicrobial resistance of lactose fermenter Enterobacteriaceae from clinical and environmental specimens.

2. Methodology

2.1. Specimens

In this study, 165 clinical and environmental samples were collected from different sources in Baghdad City. Samples collection was performed from 1st September 2014 to 1st February 2015.

2.2. Clinical Specimens

A total of 93 clinical isolates were evaluated in this study. The isolates were obtained from Ibn Al Balady Hospital, Imam Ali Hospital, Al Kindy Educational Hospital, Educational Laboratory of The Medical City and Al-Shaheed Al-Sader Hospital in Baghdad City. The strains were isolated from urine, sputum, blood, and wounds. A total of 74 urine specimens, eight sputum specimens, four blood specimens, and seven wound swabs were analyzed. Eosin methylene blue (EMB) agar and MacConkey agar were used to isolate the enteric bacteria. The plates were incubated for 24 hours at 37˚C [2].

2.3. Environmental Specimens

A total of 72 environmental specimens were collected, including 27 chicken feces, 25river water samples (Shawaka Area of the Tigris River), and 20 agriculture soils.

2.4. Collection of Water Specimens

Twenty-five water samples were obtained weekly from the Shawaka area of the Tigris River in Baghdad city. The samples were collected in duplicate from the water surface into sterile 1-L glass bottles at 8:00 am and transported to the laboratory in an ice box for bacterial analysis within two hours of collection. To isolate the enteric bacteria, the membrane filter technique was used. A volume of 60 ml of the water sample was filtered through a sterile 47-mm-diameter grid-marked membrane filter with 0.45-µm pores [14]. The membrane filters

were placed on the surface of EMB agar plates for the isolation of enteric bacteria. MacConkey agar plates are used to differentiate both lactose and non-lactose fermenters bacterial isolates.The plates were incubated for 24 hours at 37˚C.

2.5. Collection of Chicken Feces Specimens

Twenty-seven specimens of chicken feces were sampled randomly each week from a farm located in eastern Baghdad. A total of 10 g of each sample was diluted in 90 ml of sterile normal saline (0.85% NaCl) and homogenized. Then, 10 µl was placed on the surface of EMB agar plates and MacConkey agar. The plates were incubated for 24 hours at 37˚C [15].

2.6. Collection of Agriculture Soil Samples

Ten agriculture soil samples were collected randomly each week from a farm. A total of 10 g of each sample was diluted in 90 ml of sterile normal saline. Then, 10 µl of each sample was placed on the surface of EMB agar plates and MacConkey agar and incubated for 24 h at 37˚C [16].

2.7. Total Viable Count for Environmental Specimens

A serial dilution was prepared in normal saline (0.85% NaCl) and plated onto nutrient agar. The total viable count was determined using the pouring plate technique on nutrient agar and counting the colonies developed after incubation at 37˚C for 24 h [17].

2.8. Biochemical Tests

Gram negative bacteria were isolated on their respective selective and differential media and were identified on the basis of culture characteristics, including Gram reaction and biochemical tests, MacConkey agar, EMB, IMViC, Urea, and Kligler Iron Agar [2]. The automatic identification system, Vitek 2 with GN card (Gram-negative fermenter and non-fermenter bacilli), was also used.

2.9. Antibiotic Sensitivity Test

The antibiotic susceptibility profile of the Gram-negative isolates was determined using the standard Kirby-Bauer disk diffusion method [18]. These antibiotics with their respective disk concentrations are as follows: B_lactam group, including amoxicillin (25 µg), ampicillin (25 µg), ceftriaxone (10 µg), ceftazidime (10 µg), and cefepime (30 µg); aminoglycosides group, including amikacin(10 µg), gentamycin (10 µg), and tobramycin (10 µg); carbapenems group, including imipenem (10 µg) and meropenem (10 µg); monobactam group, including gaztreonam (30 µg); quinolones group, including ciprofloxacin (10 µg) and levofloxacin (5 µg); sulfonamide group, including trimethoprim + sulfamethoxazole (25 µg); and others group, including nitrofurantoin (100 µg). Bacterial culture suspension equivalents to 0.5 tube McFarland turbidity standards were spread on Muller-Hinton agar plates using sterile swabs and incubated aerobically at 37˚C for 24 hours; then, the inhibition zone diameters around the antibiotic disks were measured. The results are expressed as susceptible or resistant according to the criteria recommended by the CLSI [19].

3. Results and Discussion

The water microbiological examination is used worldwide to monitor and control the quality and safety of various water types. The present results showed the total viable count (TVC) of water was 3.26×10^4 cfu/ml (**Table 1**). While Ibrahim *et al*. report the highest TVC values were obtained in the Tigris study area from August and September, ranging from 128 to 10,000 cfu/ml [20]. The reason is due to the domestic discharges which are among the important sources of pollution of the Tigris Sanitary waters. The TVC for soil was 125.69×10^4 cfu/gram. (**Table 1**). A previous study in Egypt recorded that the TVC from agricultural soil ranged from 27×10^2 to 31×10^4 cfu/gm during the hot season [21]. On the other hand, the TVC for poultry feces was 639.43×10^4 cfu/ml (**Table 1**). Adeleke and Omafuvbe recorded the TVC for poultry feces ranged from 1.41×10^6 to 437×10^6 cfu/gm [22]. The total bacterial count of the water and soil was determined and was compared with total bacterial count of the animal feces gave a clear picture of the survival and the percentage of bacteria in environmental sample.

The most common lactose-fermenter bacterial isolates from environmental specimens were *Escherichia coli*, comprising 54.6% of the total samples, followed by *Klebsiella pneumonia*in 32.8% of samples (**Table 2**). Common water microbiological indicators are used as a marker of risk to human health include total coliform, *Escherichia coli*, enterococci, *Clostridium perfringens*, and *Pseudomonas aeruginosa* [6] [7]. Gammaproteobacteria included Enterobacteriaceae comprising 3.5% of 751 soil bacteria isolate [23]. Non-lactose fermenter, such as *Pseudomonas* spp., *Chryseomonas luteola*, *Burkholderia cepacia*, and *Aeromonas hydrophila*, are the most common bacteria in agricultural soil [21] [23]. Additionally, *E. coli* was the prevalent organism isolated from poultry feces [22]. Still *E. coli* and *K. pneumoniae* the prevalence lactose fermenters isolates from environmental sample (water and soil), and animal feces.

Clinical samples showed both *Escherichia coli* and *Klebsiella pneumonia*e as the most common species (**Table 3**). A total of 812 clinical specimens from hospitalized patients revealed that the primary bacterial species was *E. coli* from urine specimens, with *Enterobacter* spp. second and *Klebsiella* spp. sixth; however, in the blood cultures, *Enterobacter* spp. and *E. coli* were the fourth and fifth most common strains, respectively [24]. Other studies showed that the Gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* were the most common uropathogenic bacteria causing UTIs [25] [26]. Chronic surgical site infections showed *E. coli* and *Serratia* spp. as the sixth and seventh most common organisms, respectively [27]. Gram-negative organisms do not typically reside in the dry environment of normal skin [28], although occasionally, moist intertriginous areas allow for the growth of *Acinetobacter* spp. Our study showed that *Klebsiella pneumoniae* isolates were the common species isolated from the sputum followed by *Serratia marcescens*. A sputum sample from a pulmonary disease patient in the intensive care unit showed *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae* as the third, fourth and sixth most common isolates, respectively [29]. The present study also showed that *Escherichia coli* and *Klebsiella pneumonia* are the most common lactose fermenter Enterobacteriaceae iso-

Table 1. Mean ($\times 10^3$) of total bacterial counts for environmental specimens.

No.	Environmental samples	Total count ($\times 10^4$) cfu/ml or g
1	Water	3.26
2	Soil	125.69
3	Chicken feces	639.43

Table 2. Bacterial species isolated from environmental specimens.

No.	Species	Number/(%)	Environmental specimens
1	*Escherichia coli*	35 (54.6)	Water, chicken feces
2	*Klebsiella pneumoniae*	21 (32.8)	Water, chicken feces
3	*K. oxytoca*	2 (3.12)	Chicken feces
4	*Raoultella planticola*	1 (1.5)	Water
5	*Chryseomonasluteola*	1 (1.5)	Soil
6	*Burkholderia cepacia*	1 (1.5)	Soil
7	*Aeromonas hydrophila*	1 (1.5)	Soil
	Total	62	

Table 3. Bacterial species isolated from clinical specimens.

No.	Species	Number/(%)	Clinical specimens
1	*Escherichia coli*	70 (75.26)	Blood, urine, wound, pus
2	*Klebsiella pneumoniae*	14 (15.05)	sputum
3	*Enterobacter aerogenes*	6 (6.45)	Blood, urine
4	*Serratia marcescens*	2 (2.15)	sputum
5	*Citrobacter freundii*	1 (1.07)	wound
	Total	93	

lated from clinical and environmental specimens, demonstrating the importance of controlling the spread of both *E. coli* and *Klebsiella pneumonia*e and further study for virulence genetic marker.

Our study showed the antimicrobial resistance profiles for Gram negative lactose-fermenting bacteria. The most common bacterial speciesin environmental specimens was *E. coli*, and it was more multidrug resistant than clinical specimens. Furthermore, the multidrug resistance was evident for *β*-lactam drugs, aminoglycosides, monobactams, quinolones, sulfonamides and nitrofurantoin (**Figure 1**). *Klebsiella pneumonia* was the second most common species with resistance to multiple antibiotics from six antibiotic groups, mainly for clinical isolates (**Figure 2**). However, the carbapenems group, including imipenem and meropenem, remain the drugs of choice for treatment against pathogenic bacteria. The high sensitivity of the bacterial isolates to the mentioned antibiotics could be related to less frequent usage of these drugs for therapeutic purposes, therefore reducing the chance for resistance to develop.

Figure 3 displays the antibiotic activity for all bacterial species; however, the carbapenem group remains the most effective drugs. The use of antibiotics has changed the natural evolution of bacteria by reducing susceptible pathogenic populations and increasing resistant populations [30]. Large amounts of antibiotics are used for hu-

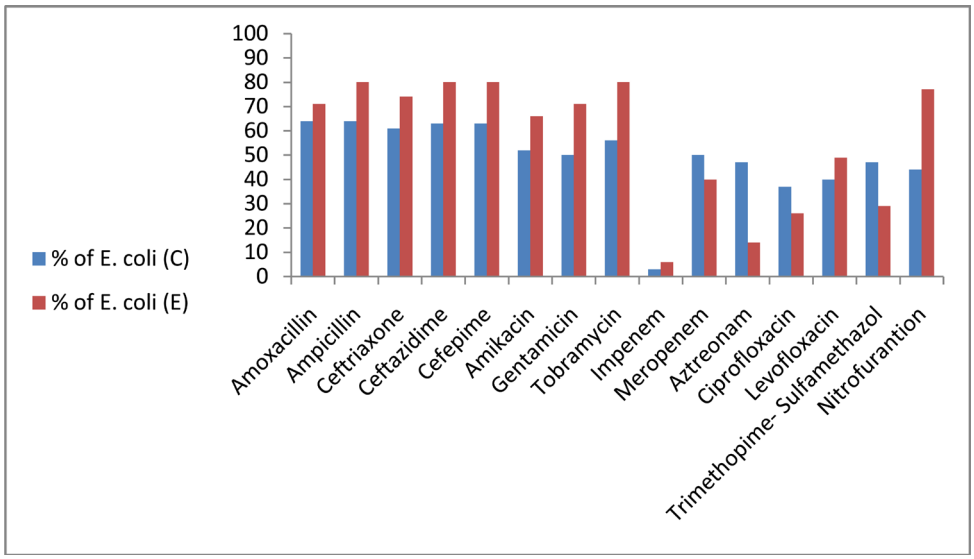

Figure 1. The percentage of antimicrobial resistance patterns of *E. coli* isolates (C: clinical specimens, E: environmental specimens).

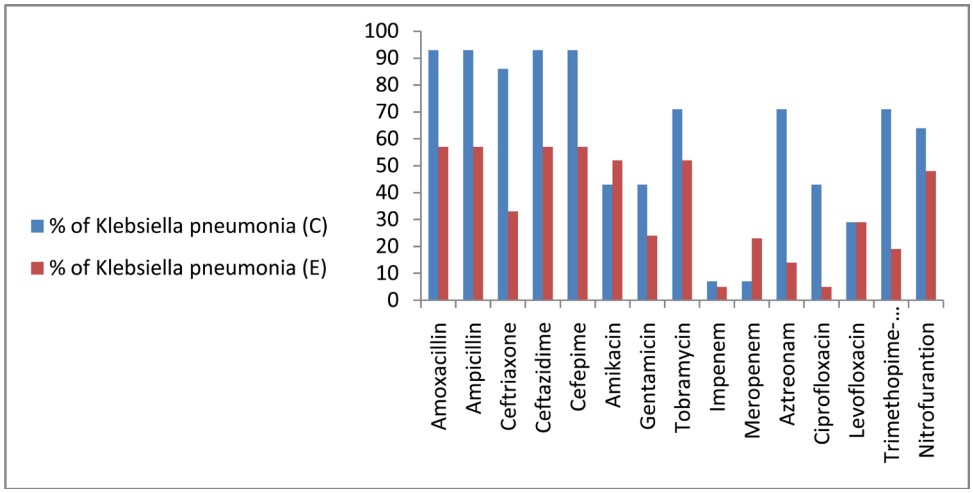

Figure 2. The percentage of antimicrobial resistance patterns of *K. pneumoniae* isolates (C: clinical specimens, E: environmental specimens).

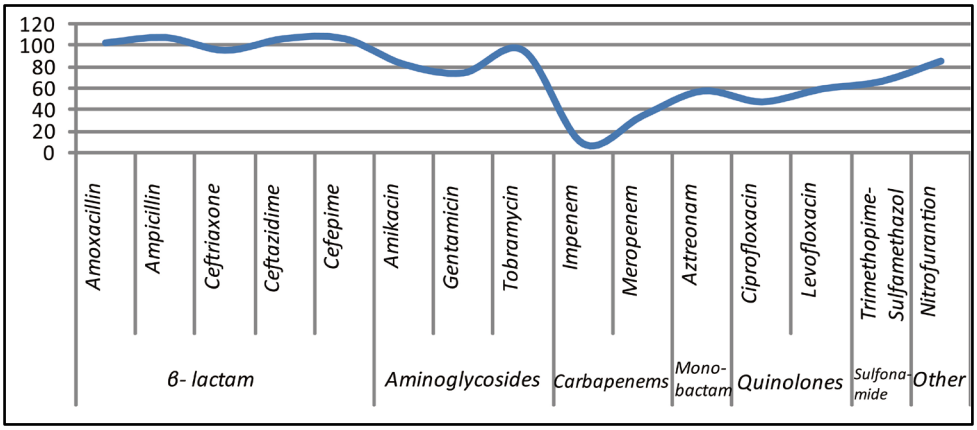

Figure 3. Comparison of antibiotic groups.

man therapy, as well as for farm animals and fish aquaculture, resulting in the selection of pathogenic bacteria resistant to multiple drugs [31]. Beta-lactam antibiotics are the largest and most commonly used group of antimicrobial agents worldwide [32]. A previous study demonstrated that these microorganisms utilize antibiotics as nutrients [33]. The genetic background of resistance mechanisms is diverse because they are present on chromosomes, plasmids, integrons and transposons [3]. Many studies suggest high levels of genetic flux between Gram-negative Enterobacteriaceae [34] that may favor plasmid exchange between Enterobacteriaceae members. Based on the results obtained in the present study, it is possible to conclude that the resistance for B-lactam antibiotic is a major problem in our community.

4. Conclusion and Recommendations

In conclusion, the most common lactose fermenter Enterobacteriaceae isolates from both the environmental and clinical samples were *E. coli* and *Klebsiella pneumonia*. The isolates exhibited multidrug resistance for B-lactam drugs (except carbapenems), aminoglycosides, monobactams, quinolones, sulfonamides and nitrofurantoin. Factors that may be associated with the transmission of resistant strains in the environment include poor hygiene, overcrowding, and antibiotic abuse. Future studies must collect more bacterial isolates from different sources in conjunction with genetic analysis. New strategies are also necessary to treat multidrug-resistant Gram negative bacteria.

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