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Antibiotics susceptibility pattern of some *Enterobacteriaceae* isolates from different clinical infectious sources

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ABSTRACT

This study was conducted to isolate some bacteria from different clinical infectious sources and determine the antibiotics susceptibility pattern usually used for the treatment of these related bacteria. From the period March 2016 to August 2016, a total of 135 different clinical specimens such as urine (50) specimens, wound (45) specimens, blood (15) specimens, and sputum (25) specimens were collected from different hospitals at Baghdad City. Iraq. *K. pneumoniae* isolates showed the following percentage of resistance; Cotrimoxazole 100 %, ceftazidime 61.1 %, aztreonam 38.9 %, piperacillin 100 %, ciprofloxacin 66.7 %, amikacin 22.3 %, gentamycin 27.8 %, Also *K. pneumoniae* isolates showed no resistance to the antibiotics imipenem and meropenem. *P. aeruginosa* isolates showed frequency resistance profile; cotrimoxazole 100 %, ceftazidime 90 %, aztreonam 70 %, piperacillin 100 %, ciprofloxacin 30%, amikacin 80 %, gentamycin 80 %, while *P. aeruginosa* isolates *showed* low resistance to the antibiotics imipenem and meropenem 20 %, 10 % respectively. *P. mirabilis* isolates showed the following percentage of resistance; cotrimoxazole 83.3%, ceftazidime 66.7 %, aztreonam 66.7 %, piperacillin 100%, ciprofloxacin 16.7 %, gentamycin 30.8 %, while *P. mirabilis* isolates showed no resistance to the antibiotics amikacin, imipenem and meropenem. The most recurrent pathogen isolated from different specimens in this study was *Escherichia coli* (31) (40.3%), followed by *Klebsiella pneumoniae* (18) (23.4%), *Pseudomonas aeruginosa* (10) (13%), *P. mirabilis* (6) (7.8%). All the isolates showed high resistance rate of 100 % to piperacillin.

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INTRODUCTION

"The *Enterobacteriaceae* are a large family of Gram-negative rods such as *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* spp. And *Pseudomonas aeruginosa* which found as part of the normal flora of human" (Pitout, 2008). They play a major role as causative human pathogens in many sites, such as the urinary tract, lower respiratory tract, blood, and abdomen in patients in community and healthcare setting (Tangden *et al.,* 2015)."The increasing prevalence of resistance to a wide range of antibiotics by these microorganisms is a major concern facing modern medicine that leading to serious public health risk in many countries (Levy, 2005; Jury *et al.,* 2005)". *Escherichia coli* is the most common Gram-negative, facultative anaerobe inhabits the human colon which responsible for infections including urinary tract, sepsis, bacteremia, neonatal meningitis and causes environmental contamination when the faecal reach to the environment (Johnson *et al.,* 2002; Dworkin *et al.,* 2006). *Klebsiella pneumoniae* is a gram-negative rod-shaped bacterium, generally encapsulated and detected in a "variety of environmental sources including water, soil, vegetation and sewage and act as an opportunistic human pathogen" (Dillon *et al.,* 2002). *Klebsiella pneumonia* is responsible for a variety infection such as nosocomial infections, meningitis, urinary and respiratory tracts, soft tissues, wounds and septicemia which colonisation rates increase in a direct proportion to the duration of the hospitalization (Dworkin *et al.,* 2006). *Proteus species* are different from other types of bacteria by their ability to swarm across an agar surface (Jacobsen *et al.,* 2008). *Proteus species* are the major cause of diseases such as infection-related kidney stone, meningitis, empyema, osteomyelitis and gastroenteritis, urinary tract, surgical wounds and lower respiratory tract infection (Mansy, 2011). *"Pseudomonas aeruginosa* is an obligate aerobic, motile, rod-shaped, a ubiquitous, Gram-negative bacterium. It is non fermenter opportunistic bacteria causing severe acute and chronic nosocomial infections associated with severe underlying medical conditions, especially in immuno-compromised patients, as well as with infected wounds, surgical wounds pneumonia, urinary tract infection and patients suffering from diabetic foot ulcers"(Vahdani *et al.,* 2012; Gellatly and Hancock, 2013). So, this study was conducted to isolate some *Enterobacteriaceae* bacteria from different clinical infectious sources and determine the antibiotics susceptibility pattern usually used for the treatment of these related bacteria.

MATERIALS AND METHODS

Samples collection, Isolation, and Identification

A total of 135 different clinical specimens such as urine (50) specimens, wound (45) specimens, blood (15) specimens, and sputum (25) specimens were collected from patients suspected of bacterial infection for the period March 2016 to August 2016 from different hospitals at Baghdad City, Iraq. Blood specimens were collected into Brain Heart Infusion container and immediately incubated at 37ºC for seven days to observe the turbidity daily. All specimens had labelled and transported by transport media with aseptic technique to the laboratory within 1-2 hour then streaked on blood agar, MacConkey agar, Eosin methylene blue (EMB) agar, Chocolate agar, Brain heart infusion {Mast group Ltd.(U.K.), Biomark Lab., Pune. (India)}and incubated at 37ºC for 24 hrs." Gram staining and morphological features of the colonies were recorded". The isolated colonies were identified by different biochemical tests according to (MacFaddinm, 2000) and the diagnosis conferred by using the VITEK 2 system.

Antimicrobial susceptibility test: Antimicrobial susceptibility testing was used on Muller-Hinton agar (Biomark Lab., Pune. India) using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline (NCCLS, 2016). The following antimicrobial agents were used (Mast group Lt. England): cotrimoxazole (TS), ceftazidime (CAZ), aztreonam (AZT), piperacillin (PRL), imipenem (IMP), meropenem (MRM), ciprofloxacin (CIP), amikacin (AK), gentamycin (GM). *Escherichia coli* ATCC 25922 was used as the control strain. At least 3-5 isolated colonies were suspended in 4-5 ml brain heart infusion broth tubes and incubated at 37°C for (18) hrs. The turbidity of the actively growing broth culture suspensions had adjusted to 0.5 McFarland standard. A sterile cotton swab was dipped into the adjusted suspension, then rotated several times firmly on the inside wall of the tube to remove excess inoculum from the swab. The entire surface of a Mueller–Hinton agar plate was streaking with the dipping swab. "The streaking was repeated two more times, and the plate was rotated approximately 60 degrees each time" to ensure the distribution of inoculum and allowed drying for 15-20 min. At room temperature before the application of the antibiotic discs. The antibiotic discs were applied to the surface of the inoculated agar plates and incubated overnight at 37°C. The diameter of the zone of growth inhibition was measured and compared to the chart provided by the National Committee for Clinical Laboratory Standards (NCCLS, 2016).

Statistical Analysis

Descriptive statistics and tables were used. The incidence of resistance of isolates of bacteria to antibacterial agents was calculated as the number of resistant isolates divided by the number of total isolates examined, multiplied by 100.

RESULTS

The result of Gram staining showed that the samples were Gram-negative, Gram-positive, and Candida. The results of cultured samples showed that from (135) isolates; (77) (57%) isolates Gram-negative bacteria as single pathogens growth identified as; the most recurrent pathogen isolated from different specimens was *Escherichia coli* 31 (40.3%), followed by *Klebsiella pneumonia* 18 (23.4%), *Pseudomonas aeruginosa* 10 (13%), *P. mirabilis* 6 (7.8%) and 12 (15.6%) other Gram-negative bacteria. Single isolates Gram-positive bacteria 18 (13.3%), 9 (6.7%) isolates Candida and 25 (18.5%) mixed growth, while 6 (4.4%) cultured specimens showed no growth. Other Gram-negative bacteria, Gram-positive isolates, Candida and mixed growth isolates were excluded. The main isolates were *Escherichia coli* 31(15, 8, 2, 6) from urine, wound, blood, and sputum respectively. The second isolates were *Klebsiella pneumonia* 18 (8, 3, 1, 6) from urine, wound, blood, and sputum respectively. While *P. aeruginosa* 10 (4, 6) and *P. mirabilis*

Table 1: Isolated bacteria from clinical specimens

Type of bacteria	Clinical specimens				
	urine	wound	blood	sputum	Total (%)
Escherichia coli	15	8	2	6	31 (40.3)
K. pneumoniae	8	3	1	6	18 (23.4)
P. aeruginosa	4	6	-	-	10 (13)
Proteus spp.	4	2	-	-	6 (7.8)

Table 2: Antibiotics resistance profile of some gram-negative bacteria

Antimicrobial drug	*E. coli* (%)	*K. pneumoniae* (%)	*P. aeruginosa* (%)	*P. mirabilis* (%)
cotrimoxazole	25 (80.6 %)	18 (100 %)	10 (100 %)	5 (83.3%)
ceftazidime	27 (87.1 %)	11 (61.1 %)	9 (90 %)	4 (66.7 %)
aztreonam	29 (93.5 %)	7 (38.9 %)	7 (70 %)	4 (66.7 %)
piperacillin	31 (100 %)	18 (100 %)	10 (100 %)	6 (100%)
imipenem	-	-	2 (20 %)	-
meropenem	-	-	1 (10 %)	-
ciprofloxacin	24 (77.4 %)	12 (66.7 %)	3(30%)	1 (16.7 %)
amikacin	8 (25.8 %)	4 (22.3 %)	8 (80 %)	-
gentamycin	9 (29.1 %)	5 (27.8 %)	8 (80 %)	2 (33.3 %)

Table 3: Antibiotics sensitivity profile of some gram-negative bacteria

Antimicrobial drug	*E. coli* (%)	*K. pneumoniae* (%)	*P. aeruginosa* (%)	*P. mirabilis* (%)
cotrimoxazole	6 (19.4 %)	-	-	1 (16.7 %)
ceftazidime	3(9.6 %)	6 (33.3 %)	1 (10 %)	2 (33.3 %)
aztreonam	1 (3.2 %)	11(61.1 %)	2 (20 %)	2 (33.3 %)
piperacillin	-	-	-	-
imipenem	31 (100 %)	18 (100 %)	8 (80 %)	6 (100%)
meropenem	31 (100 %)	18 (100 %)	9 (90 %)	6 (100%)
ciprofloxacin	7(22.5%)	6 (33.3 %)	7 (70 %)	5 (83.3%)
amikacin	21 (67.7 %)	11 (61.1 %)	2 (20 %)	6 (100%)
gentamycin	22(70.9 %)	13 (72.2 %)	1 (10 %)	4 (66.7 %)

6 (4, 2) isolates were yielded from urine and wound only. The largest specimens were within the urine 15 (48.4%) from the total isolates 31 of *Escherichia coli* followed by *Klebsiella pneumonia* 8 (44.4%) from the total isolates 18, while 4 (40%) from the total isolates 10 and 4(66.7%) from the total isolates 10, 6 of *Pseudomonas aeruginosa* and *P. mirabilis* respectively (table 1).

The results had shown various grade of sensitivity and resistance profile as followed: From table 2, table 3 and figure 1 *E. coli* isolates showed frequency resistance profile; cotrimoxazole 80.6 %, ceftazidime 87.1%, aztreonam 44.6 %, piperacillin 100 %, ciprofloxacin 36.9 %, amikacin 12.3 %, gentamycin 29.1 %, while *E. coli* isolates *showed* no resistance to the antibiotics imipenem and meropenem. *K. pneumoniae* isolates showed the following percentage of resistance; Cotrimoxazole 100 %, ceftazidime 61.1 %, aztreonam 93.5 %, piperacillin 100 %, ciprofloxacin 77.4 %, amikacin 25.8 %, gentamycin 27.8 %, Also *K. pneumoniae* isolates showed no resistance to the antibiotics imipenem and meropenem.*P. aeruginosa* isolates showed frequency resistance profile ; cotrimoxazole 100 %, ceftazidime 90 %, aztreonam 70 %, piperacillin 100 %, ciprofloxacin 30%, amikacin 80 %, gentamycin 80 %, while *P. aeruginosa* isolates showed low resistance to the antibiotics imipenem and meropenem 20 %, 10 % respectively. *P. mirabilis* isolates showed the following percentage of resistance; cotrimoxazole 83.3%, ceftazidime 66.7 %, aztreonam 66.7 %, piperacillin 100%, ciprofloxacin 16.7 %, gentamycin 30.8 %, while *P. mirabilis* isolates *showed* no resistance to the antibiotics amikacin, imipenem and meropenem.

DISCUSSION

Infection by Gram-negative pathogens is a main difficult that usually occurs in patients suffering from clinical infectious. The principal in the management of these infections is the suitable use and choice of antimicrobial therapy. Therefore, the controlling of this infection is very important to prevent significant rates of drug resistance among different gram-negative bacteria (Church *et al.,* 2015). The results of this study inducted that the most recurrent pathogen isolated from different specimens was *Escherichia coli* 31(40.3%), followed by *Klebsiella pneumonia* 18 (23.4%), these results were in agreement with Cyprien *et al.,*

(2015) who pointed that *E. coli* was the most frequent pathogen isolated from urine specimens followed by *Klebsiella pneumoniae*. Also, Israa and Tuqa, (2015) who refer that the most common bacterial isolates from the clinical and environmental samples were *Escherichia coli* and *Klebsiella pneumonia*, respectively. While Olayinka *et al.,* (2010) refer that the highest rate of infection with *P. aeruginosa* was from infections at the urinary tract. The clinical isolates that obtained from clinical specimens showed significant rates of drug resistance among different gram-negative bacteria.

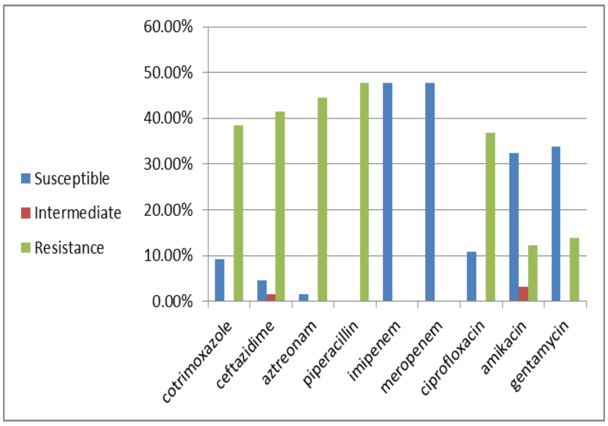

Figure 1: Antibiotic susceptibility test of E. coli to different antibiotics

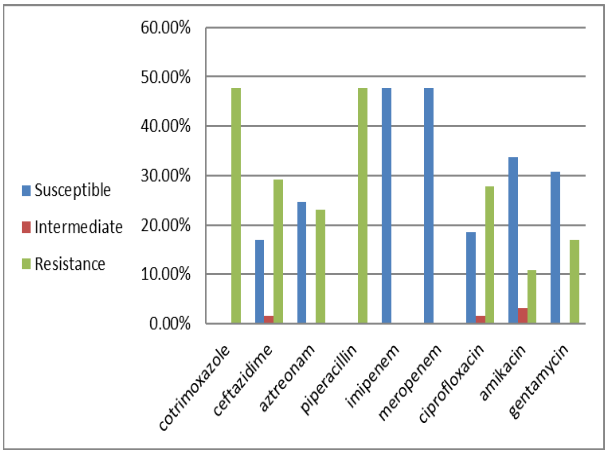

Figure 2: Antibiotic susceptibility test of K. pneumoniae to different antibiotics

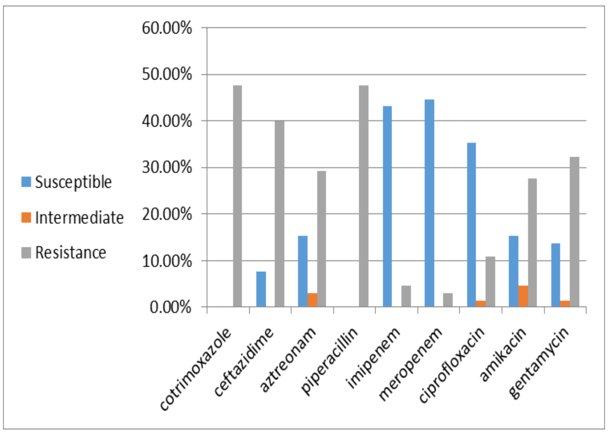

Figure 3: Antibiotic susceptibility test of P. aeruginosa to different antibiotics

The results of antibiotic susceptibility test of *E. coli* to nine different antibiotics showed frequency resistance profile table 2 and figure 1. The study showed high resistant to cotrimoxazole 80.6 %, these results agree with the study done by Tärnberg, (2012) who refer that the isolated bacteria were resistant to this antibiotic and the isolates showed multidrug resistance. Also, the results of this study agree with the study done by Al-Nedawy, (2005) who found that the resistant rate of *E. coli* isolates was 73.33%. From another side the isolates showed high resistant to the antibiotics ceftazidime (87.1 %), Aztreonam (93.5 %) and piperacillin (100 %) which agree with the study done by Dua'a, (2011) who refer that the isolated bacteria showed resistant rate to the ceftazidime, Aztreonam, and piperacillin; (46.6%), (60 %) and (93.3%) respectively. The current study isolates of *E. coli* did not show any resistance to the antibiotics Imipenem and meropenem (carbapenems group) which agree with the study done by Al-Garawy, (2009). This finding might be due to the limited use of these antibiotics as routine antibiotic treatment. The isolates showed the resistance percentage to the ciprofloxacin (Quinolones group) 77.4 %. This result was agreeing with the study done by Al-Mhemedawy, (2000) where *E. coli isolates* showed a resistance percentage of 47.3%. This group of antibiotics inhibits the synthesis of bacterial DNA by inhibiting the DNA gyrase enzyme and the process of supercoiling of the DNA strips (Hardy *et al.,* 2005). In this study, the isolates of *E. coli* showed low resistance to Aminoglycosides (amikacin and gentamycin) which disagree with the study done by Andrasevic and Dowzicky, (2012) who found that *Enterobacteriaceae* isolates were resistant to amikacin and gentamycin (Aminoglycosides group). Farajnia *et al.,* (2009) reported that the inappropriate use, misuse and under-use of antibiotics in medicine are responsible for the increased resistance to the antibiotics worldwide. This fact becomes true when the transfer of resistant occurs in surroundings such as hospitals where the human residents are at risk. The antibiotics resistance of *K. pneumoniae* in this study also showed frequency resistance profile against the following antibiotics table 2 and figure 2: The study showed resistant to cotrimoxazole 18 (100 %), these results agree with the study done by Sandeep *et al.,* (2017) who found that high antibiotic resistance to cotrimoxazole 143(70.8 %). Another study by Giannoula *et al.,* (2013) who refer that the susceptibility rate of trimethoprim-sulfamethoxazole was varied from 15 to 60%. Also, the results of this study show that the antibiotic ceftazidime was effective against *Klebsiella pneumonia* with 33.3 % sensitivity. These results were in agreement with the study done by Samah *et al.,*

(2017) who found that ceftazidime the most effective antibiotic against *Klebsiella* species after imipenem.

"Pavani and Fathima, (2015) found that the percentage of resistant of isolates to aztreonam in their study 92% (32% out of 200 isolates)", while the present study revealed that the percentage of resistant of isolates 38.9 %. Also, in this study, the maximum resistance of *Klebsiella pneumonia* isolates 100 % to piperacillin which agrees with the study done by Sandeep *et al.,* (2017) who found that all isolates (117) resist to piperacillin. The current study isolates of *Klebsiella pneumonia* did not show any resistance to the antibiotics imipenem and meropenem (carbapenems group) which agree with the study done by Sandeep *et al.,* (2017). Other study revealed that the percentage of resistant isolates to imipenem and meropenem 59.4% and 29 % respectively. The isolates showed the resistance percentage to the ciprofloxacin (Quinolones group) 66. 7% and susceptibility 33.3 % table 3. These results disagree with the study done by Giannoula *et al.,* (2013) who refer that the antimicrobial susceptibility of *Klebsiella* species isolates to ciprofloxacin from 53 to 100%. In this study the isolates of *K. pneumoniae* showed low resistance to Aminoglycosides (amikacin and gentamycin) which disagree with the study done by Aisha *et al.,* (2013) who found high resistance of their isolates to gentamycin reach to 78 %, while agreeing with the study done by Sandeep *et al.,* (2017) who found that low antibiotic resistance to amikacin 5%. From table 2 and figure 3 the study showed high resistant to cotrimoxazole, these results were agreeing with the study done by Eucharia, (2013) and Rosas *et al.,* (2015) who refer that the isolated bacteria were resistant to this antibiotic, while Ehsan, (2016) found that this antibiotic had high activity against *P. aeruginosa*. Also, the isolates showed high resistant to piperacillin and ceftazidime. This result was in agreement with the study done by Cyprien *et al.,* (2015) who found that 33% of the *Pseudomonas* species showed resistance to antipseudomonal β-lactams (piperacillin and ceftazidime). The current study isolates of *P. aeruginosa* showed the percentage of resistant to aztreonam 7 (70 %), this result was in agreement the study done by Rama *et al.,* (2012) who found that the percentage of resistant to this antibiotic between 90 to 100 %. All the strains of *P. aeruginosa* in this study showed low resistant to imipenem and meropenem 20 % and 10 % respectively. While Rama *et al.,* (2012) refer that their isolates were sensitive to imipenem. Gehan et al*. did another study.,* (2011) found that strains varied in its sensitivity to imipenem (18.8%), meropenem (20.3%). The isolates showed the resistance percentage to the ciprofloxacin (Quinolones group) 30 %. Also, the same study by Gehan, *et al.,* (2011) found that the resistance percentage to the ciprofloxacin was17.4%. Studies from Belgium, Iran, and Turkey documented the antimicrobial resistance percentage to ciprofloxacin were 76%, 29%, and 21%, respectively (Glupczynski *et al.,* 2001; Kucukates, 2005; Hadadi *et al.,* 2008).

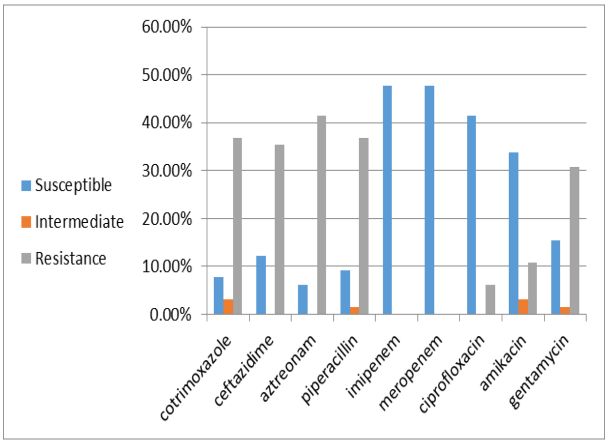

Figure 4: Antibiotic susceptibility test of P. mirabilis to different antibiotics

In this study, the isolates of *P. aeruginosa* showed the resistance percentage to Aminoglycosides (amikacin and gentamycin) 80 % respectively which agree with the study done by Kehinde *et al.,* (2004) who found that their isolates show highly resistant to gentamycin. Another study was done by Wardhana *et al.,* (2017) who referred that their isolates were highly sensitive to amikacin and gentamycin from the aminoglycoside antimicrobial spectrum. From table 2 and figure 4 the result of this study showed that imipenem, meropenem, and amikacin were the most effective antibiotics against *Proteus mirabilis*. This result agrees with the study done by Saleh and Hatem, (2013) and Patrick *et al.,* (2010) who found that the antibiotic imipenem was very effective against *Proteus* spp. Also, the isolates show high resistance to cotrimoxazole 5 (83.3%) which agree with the study done by Patrick *et al.,* (2010) who found high antimicrobial resistance to co-trimoxazole 81 %. The *Proteus mirabilis* isolates in the present study were found to have high antimicrobial resistance against piperacillin (100%). This result was disagreeing with the study done by Cyprien *et al.,* (2015) who found that the isolates of *Proteus* spp. were susceptible to piperacillin. The present study of *P. mirabilis* isolates showed the percentage of resistant to aztreonam 4(66.7 %), this result was in agreement the study was done by Rama *et al.,* (2012) who found that the percentage of resistant to this antibiotic 100 %. The isolates of Proteus *mirabilis* showed low resistance percentage to the ciprofloxacin (Quinolones group) 16.7 %. This result was in agreement with the study done by Wendy *et al.,*

(2018) who found that all isolates of *Proteus mirabilis* were susceptible to ciprofloxacin. In this study, the isolates of *P. mirabilis* showed low resistance percentage to aminoglycosides (gentamycin) 33.3 %. This result was in agreement with the study done by Makled and Alghamdi, (2006) who referred that aminoglycosides were used in the treatment of Proteus *mirabilis* isolates are still effective.

CONCLUSION

The most recurrent pathogen isolated from different specimens in this study was *Escherichia coli* (31) (40.3%), followed by *Klebsiella pneumoniae* (18) (23.4%), *Pseudomonas aeruginosa* (10) (13%), *P. mirabilis* (6) (7.8%). All the isolates showed high resistance rate of 100 % to piperacillin. While the most effective antimicrobial agents against the isolates were imipenem, meropenem followed by amikacin and gentamycin.

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