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(RESEARCH ARTICLE)

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Molecular detection of extended spectrum β-lactamase in gram negative bacteria isolated from intensive care unit patients in Wasit province, Iraq

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Abstract

Intensive care units (ICU) are epicenters for the emergence of antibiotic-resistant Gram negative bacteria and multi-resistant Gram positive infections, largely due to the inappropriate use of antimicrobials. In this study, a total of 100 clinical specimens (urine, sputum and pus) were collected from patients admitted in the ICU. Results showed eighty two were positive growth culture. The findings of the disc diffusion method used to test the isolates for antibiotic susceptibility showed that Amikacin was the most effective antibiotic against *klebsiella pneumonia* and *pseudomonas aeruginosa.* Phenotypically survey of ESBLs for all isolates were ESBL producers, of these, 13(68.4%) isolates were determined as ESBLs-producers following confirmatory testing to be ESBLs producers. Whereas, genotypically no isolate had ESBLs producers *CTX-M*-type ESBL. The prevalence of *TEM, SHV*, and *OXA*-1 were as follow: bla_{SHV} 3(23 %). While bla_{TEM} and bla_{OXA}-1genes were absent among all isolates. Phenotypically survey of ESBLs, for (20) isolates *pseudomonas aeruginosa* 20(100%) isolates were ESBLs producers of these, 12(60%) isolates were determined as ESBLs-producers following confirmatory testing to be ESBLs producers. While genotypically isolates were ESBL producers 1(8.3%) of the isolates were ESBLs producers *CTX-M*-type ESBL was the most prevalent *CTX-M*2 was 1(8.3%) , *CTX-M*1 and *CTXM9* were absent among all isolates. The prevalence of *TEM, SHV*, and *OXA*-type1 were as follow: bla_{SHV} 4(33%). While bla_{TEM} and bla_{OXA}-1 genes were absent among all isolates. Phenotypically survey of ESBLs, for (17) isolates E. coli 17(100%) isolates were ESBLs producers of these, 10(58.8%) isolates were determined as ESBLs-producers following confirmatory testing to be ESBLs producers. Whereas, Genotypically no isolate ESBLs producers *CTX-M*-type ESBL, prevalence of *TEM, SHV,* and *OXA*-1 were as follow: bla_{SHV} 1(10%),blaOXA-1 1(10%) . While bla_{TEM} gene was absent among all isolates.

Keywords: Antibiotic susceptibility; ESBLs; Gram negative bacteria; Intensive care unit

1. Introduction

Intensive care units (ICUs) are important departmants at the hospital for nosocomial infections. Although an ICU has 5%– 10% of the hospital beds, 25%–50% of the nosocomial infections originate from the ICU [1]. Both ventilator-related pneumonia (VRP) and catheter-related urinary tract infections (CRUIs) are the most common infections in the ICU [2]. Many risk factors are responsible for nosocomial infection in the ICU [3]. Some of the risk factors are related to the patient, whereas the others are related to the external factors. It is known that improving the risk factors decreases the infection, mortality, morbidity, and cost [2,4]. The environmental conditions affect the infection rate in the ICU [5]. Hospital-acquired infections (HAI) have been recognized for over a century as a critical problem affecting the quality of healthcare, and they constitute a major source of adverse healthcare outcomes [6]. The emergence of multidrug-resistant bacteria (MDRB) has become a public health problem, creating a new burden on medical care. In addition to

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this, ICU patients have an increased risk of infection due to their underlying diseases or conditions, impaired immunity, and exposure to multiple invasive devices (mechanical ventilation, central venous catheters (CVC), and urinary tract catheters), the incidence of ICU-HAI is 5–10-times higher than HAI rates in general wards [7]. Intensive care units (ICU) are epicenters for the emergence of antibiotic-resistant Gram negative bacteria and multi-resistant Gram positive infections, largely due to the inappropriate use of antimicrobials [8]. The aim was presented to detect spread of bacterial isolates from patients in ICU in Kut City, Wasit Province, Iraq, and to characterize it at the level of molecular analyses of its Phylogenic and antimicrobial resistance genes.

2. Material and Methods

A cross-sectional study was done in the intensive care unit (ICU) of the Alzahraa, Alkarama hospitals from the 3rd October 2021 to 20th February 2022. A total of 100 clinical samples, including: urine, sputum and pus culture media such as mannitol salt agar, MacConkey agar, blood agar, and chocolate agar. The growth showed different bacterial colonies whose morphological and biochemical characteristics were tested. Then DNA was extracted; purity and concentration were confirmed with Nanodrop. The purity of gram negative bacteria (1.8-2), and the concentration was between 50-360 ng/µl.

Table 1 Primers' sequence of gram-negative bacteria

MultiplexPCR pool	Primers	Sequences (5'-3')	Size(bp)	References
MultiplexI TEM, SHV and OXA -1 Like	Multi TSO-Tfor	CATTTCCGTGTCGCCCTTATTC	800	(Dallenne *et al.*, 2010)
	Multi TSO-Trev	CGTTCATCCATAGTTGCCTGAC		
	Multi TSO- S-for	AGCCGCTTGAGCAAATTAAAC	713	
	Multi TSO-Srev	ATCCCGCAGATAAATCACCAC		
	Multi TSO-Ofor	GGCACCAGATTCAACTTTCAAG	564	
	Multi TSO-Orev	GACCCCAAGTTTCCTGTAAGTG		
Multiplex-II: CTX-M group 1, group 2 and group 9	Multi CTXMGP1 –for	TTA GGA ART GTG CCG CTG YAb	688	(Ugwu *et al.*, 2020)
	Multi CTXM GP1-2-rev	CGATATCGTT GGT GGTRCCATb		
	Multi CTXMGP 2-for	CGTTAACGGC ACGATGAC	404	
	MultiCTX MGP1-2- rev	CGATATCGTT GGTGGTRCCATb		
	Multi-CTXMGP9-F	TCAAGCCTGCCGATCTGGT	561	
	Multi-CTXMGP9-R	TGATTCTCGCCGCTGAAG		

Production of ESBLs was initially phenotypically discovered using the disk diffusion method (screening test), and was then verified using the double disc synergy test (DDST) [9]. Wheraes an isolate is considered tobe ESBL producer in the case when it shows resistance to one or more of the 3rd. Generation cephalosporins asfollows: cefotaxime (≤ 27mm); ceftazidime (≤ 22mm); ceftriaxone (≤ 25mm) and aztreonam (≤ 27mm).

Table 2 Thermal cycling program for multiplex pool.1 CTX group

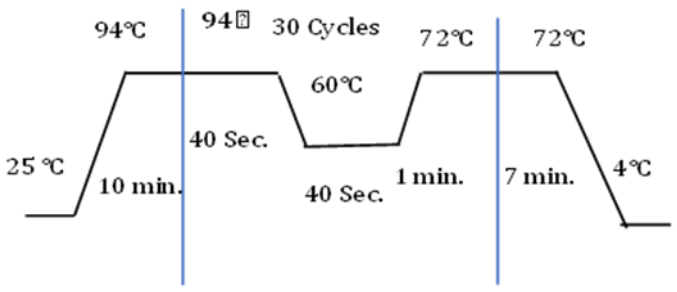
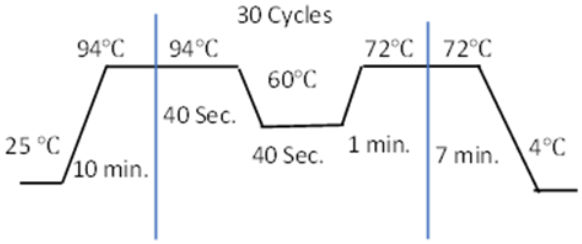
Genes	PCR cycling profile	Products size
***bla*$_{ctx1,2,9}$**		Ctx1 688 Ctx2 404 Ctx9 561

Table 3 Thermal cycling program for multiplex pool.2 TSO

Genes	PCR cycling profile	Products size
bla*$_{TEM}$, *SHV,andOXA-1		*TEM* 800 *SHV* 713 *OXA-1* 564

According to who adapted this protocol as a reliable and efficient tool for the identification via multiplex PCR assays of the most common encoding of the beta-lactamase genes for gram negative bacteria? Gel Electrophoresis and Documentation [10] and finally the data's statistical analysis has been carried out with the use of SAS (Statistical Analysis System - version 9.1) [11].

3. Results and discussion

The current study was conducted on a total of one hundred clinical specimens (urine, sputum and pus) were collected from patients admitted in the ICU. Eighty two were positive growth culture distributed according to the patient's age, the highest incidence was among 20-29 age groups with (25.6 %). While the lowest incidence was among (50-59) and (70-79) age group (3.6%),(3.6%) respectively ,the mean ±SD of age was 15.556 ranging from 1 years to 90 years.

Eighty two pure positive culture, 30(36.5%) were female of patients 52(63.5%) were male and admitted to the intensive care unit distributed 69 (84.1%) gram-negative bacteria. while gram positive 13(15.8%) .As shown in Table (4).

Table 4 Bacteria distribution according to specimens

Culture result	Urine No(%)	Sputum No(%)	Pus No(%)
Klebsiella pneumoniae	3 (3%)	11 (11%)	5 (5%)
Klebsiella oxytoca	0 (0%)	1 (1%)	0 (0%)
E. Coli	11 (11%)	3 (3%)	6 (6%)
P. aeruginosa	4 (4%)	9 (9%)	7 (7%)
Proteus mirabilis	0 (0%)	0 (0%)	5 (5%)
Acinetobacter baumannii	0 (0%)	2 (2%)	0 (0%)
Serratia marcescens	1 (1%)	0 (0%)	0 (0%)
Burkholderia cepacia group	0 (0%)	1 (1%)	0 (0%)
Pantoea spp	0 (0%)	0 (0%)	1 (1%)
Staph.aureus	2 (2%)	3 (3%)	6 (6%)
Staph heamolytius	0 (0%)	0 (0%)	2 (2%)
Total	21	30	32

Bacterial isolates that were obtained from the clinical specimens have been initially characterized based on the cultural morphology as well as the biochemical tests. Results of culture showed colonies on MacConkey agar pink color with

precipitation of bile salt around colonies, the refers to *Escherichia coli* isolates while results of biochemical tests for *Escherichia coli* were had given positive test for catalase, indole, methyl red, but negative for oxidase, voges-proskauer, simmon citrate, and TSI test showed A/A with gas , without H_2S. This results diagnostic for *Escherichia coli* .Similar results was recorded by [12–14].

Results of identification of *klebsiella pneumoniae* were showed pink lactose fermenter mucoid colonies on MacConkey agar. Positive test for urease, voges-proskauer, simmon citrate , but negative for oxidase, methyl red, motility test, indole and TSI test showed A/A with gas , without H_2S . Further confirmation done using Vitek2. Similar results were recorded by [15, 16].

Results of *Acinetobacter baumannii* showed nonmotile and give negative result to oxidase, indole, methel red, voges proskauer and avirable to urea. All isolates were positive to catalase , simmons citrate and triple-sugur-iron test was alkaline / no change, these results are identical with those obtained by [17,18]. The results of *Pseudomonas aeruginosa* showed that all isolates had given negative result for indole, methyl red, voges proskauer and urease test also, citrate assimilation was positive, motility was positive and TSI alkaline / alkaline or Alkaline / no change with no production of H_2S and gas and isolated from cetrimide agar colonies appeared mucoid, smooth in shape, fruity odour, fluorescent green, and creamy pigments these results agreed with what mentioned by [13,18–20].

The results of *Proteus mirabilis* appear as bacilli, rapidly motile by flagella. Swarming phenomena on blood agar plate. It is non-lactose fermenter so it gives a pale colony on macConky agar. Negative test for indole and voges-proskauer but positive test for urease, simmon ،s citrate , H_2S is produced ,nitrate reduction motility and methyl reed .Similar finding was recorded by [15,18].

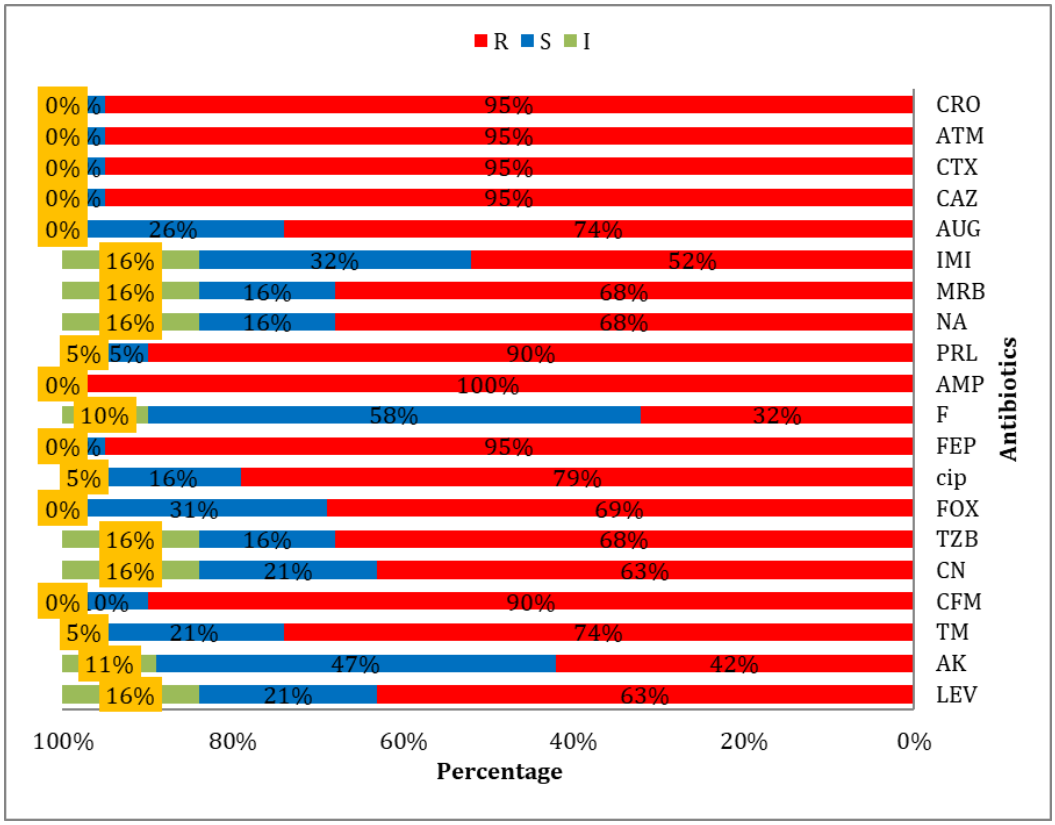
The results of *Serratia. marcescens*, showed that isolate had given result for indole, urease test , citrate assimilation , motility and catalase positive . Methyl red, voges proskauer and oxidase negative and TSI alkaline / acidic or acidic / acidic with no production of H_2S and gas.The results of *Burkholderia* cepacia complex showed that isolate had given a result at negative for indole. But positive for methyl red, voges proskauer, urease test, citrate assimilation, motility, catalase and oxidase, TSI alkaline / acidic with no production of H_2S and gas.

The results of *Pantoea spp* showed that isolate had given result for indole methyl red, urease test, motility,and oxidase negative. Voges-proskauer citrate assimilation, catalase positive, TSI alkaline / acidic with no production of H_2S and gas. The results *Enerobacter cloacae* indicated that isolate had given result for indole, methyl red,urease test , motility and oxidase negative. Voges-proskauer, citrate assimilation and catalase was positive. TSI alkaline / acidic with no production of H_2S and gas. The results of *staphylococcus aureus* appeard that isolates had given result for indole was negative , methyl red was positive , voges-proskauer was positive , urease test was positive , citrate assimilation was positive, motility was negative and TSI acidic / acidic with no production of H_2S and gas , catalase was positive , coagulase was positive and oxidase was positive this results are consistent with findings from other Iraqi studies [20,21]. Also similar result was recorded by [21–23]. On blood agar *staphylococcus haemolyticus* fermenters as yellow colonies. Further biochemical tests were necessary for identification of *staphylococcus haemolyticus* from other species, all isolates were positive to catalase test, but negative for coagulase and oxidase [24]. The results of *Enterococcus faeciu* showed that isolate had given negative results for indole, urease test, citrate assimilation, motility, catalase and oxidase. But positive result for voges-proskauer. TSI acidic / acidic with no production of H_2S and gas.

3.1. Antibiotic susceptibility test of *klebsiella pneumonia* isolates

The maximum resistance level of study to Ampicillin was 100 % followed by Ceftriaxone , Azitreonam ,Cefotaxime , Ceftazidime , and Cefepime with (95%) respectively , (Cefixime and Piperacillin)(90%) respectively , (Ciprofloxacin 79%), (Trimethoprime and Amoxicillin_clavulanic acid) (74%) respectively , (Cefoxitin 69 %),(Meropenem ,Nalidixic acid ,and Piperacillin _tazobactam) (68%)respectively, (Gentamicin and Levofloxacin) (63%), (Imipenem 53%). As shown in figure (1).

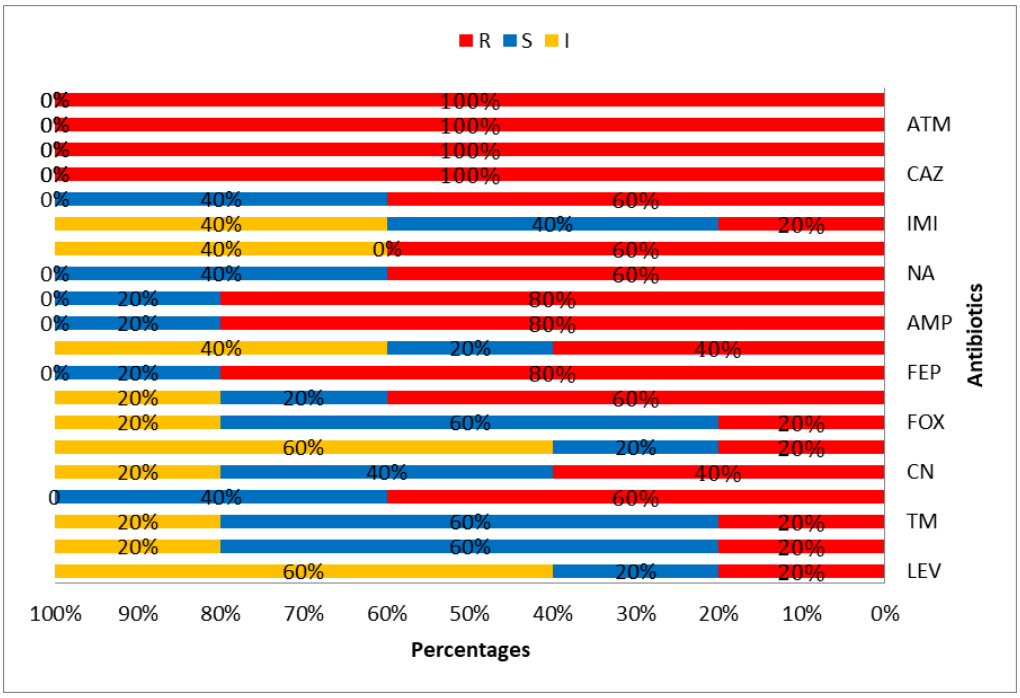
The results revealed maximum resistant to Ampicillin was (100 %). These data are in agreement with the study performed by [25,26]. Resistance percentage of *klebsiella pneumoniae* to ciprofloxacin (79%) was agreement with the study performed by [27,28] and [16], Study revealed that amoxicillin -clavulanic acid (77%) by [16] agreed with this results. The maximal *klebsiella pneumoniae sensitivity* has been to (amikacin 58 %), and (nitrofurantoin 47 %), which in agreement with [16,29]. The medical literature uses the terms multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria to describe the various patterns of resistance seen in healthcare-associated, antimicrobial-resistant bacteria. It was divided according to the criteria by [30].The percentages were as follows MDR were (47%), XDR were (21.5%) and PDR were (31.5%).



CRO = Ceftriaxone, AZT= Aztreonam, CTX = Cefotaxime, CAZ = Ceftazidime, AUG=Amoxicillin-clavulanic acid, IMI=Imipenem, MRB= Meropenem, NA= nalidixic acid, PRL=piperacillin, AMP = Ampicillin, F = Nitrofurantoin, FEP=Cefepime, CIP=Ciprofloxacin, FOX = Cefoxitin, TZP=piperacillin-tazobactam, CN = Gentamicin,CFM = Cefixime, TM=Trimethoprime, AK=Amikacin ,LEV=levofloxacin

Figure 1 The percentage of antibiotics susceptibility profiles of *K.pneumonia*

3.2. Antibiotic susceptibility test of *Pseudomonas eruginosa* isolates



CRO = Ceftriaxone, AZT= Aztreonam, CTX = Cefotaxime, CAZ = Ceftazidime, AUG=Amoxicillin-clavulanic acid, IMI=Imipenem, MRB= Meropenem, NA= nalidixic acid, PRL=piperacillin, AMP = Ampicillin, F = Nitrofurantoin, FEP=Cefepime, CIP=Ciprofloxacin, FOX = Cefoxitin, TZP=piperacillin-tazobactam, CN = Gentamicin,CFM = Cefixime, TM=Trimethoprime, AK=Amikacin ,LEV=levofloxacin

Figure 2 The percentage of antibiotics susceptibility profiles of *P. aeruginosa*

The maximum resistance level of study for (Azitreonam ,Ceftriaxone ,Cefotaxime ,and Ceftazidime) (100 %)respectively followed by (Cefepime, Piperacillin ,and (Ampicillin) (80%)respectively followed by (Meropenem , Nalidixic acid , Ciprofloxacin and Amoxicillin - clavulanic acid and Cefixime) (60%)respectively . As shown in figure (2).

These results agree with study in Wasit [31,32] which found to be highly resistant to (ceftazidime, ceftriaxone, azitreonam and cefotaxime) (100%) respectively. The maximal *pseudomonas aeruginosa* sensitivity has been to (amikacin, trimethoprime and cefoxitin) (60%) respectively. In *pseudomonas aeruginosa*, intrinsic antibiotic resistance is mediated by a decrease of outer membrane permeability, the expression of MDR, XDR and PDR efflux pumps, as well as the synthesis of antimicrobial inactivating enzymes (Hall *et al*., 2018). In our study, it was observed that the percentages (, MDR) were (70%), XDR were (15%) and PDR were (15%), as shown in the figure (3_9). This increase in percentage leads to multiple drug resistance of isolates to antimicrobials because of the province the outer membrane's permeability barrier antibiotics out of the bacterial cells

3.3. Screening and confirmation of ESBLs' production for *K. pneumoniae*

Presumptive ESBLs-producing *K. pneumoniae* were detected in 19 clinical specimens , were initially screened for ESBLs and result 18(94.7%). Of these, 13(68.4%) isolates were determined as ESBLs-producers following confirmatory testing to be ESBLs producers by CLSI complex disk detection (CLSI-CDD) method (modified double-disc synergy test). Resistance of *K. pneumoniae* to cephalosporins (third generation) and monobactams were as the following: cefotaxime: 94.7%, ceftazidime 94.7%, ceftriaxone 94.7%, and aztreonam: 94.7%

3.4. Screening and confirmation of ESBLs' production for *P. aeruginosa*

The clinical isolates 20 of *P. aeruginosa* for ESBL production , were initially screened for ESBLs and result 20(100%) and the confirmatory assay result was 12 (60%) by (modified double-disc synergy test). Resistance of *P. aeruginosa* to third generation cephalosporins and monobactams were as the following: cefotaxime: 90%, ceftazidime: 95%, ceftriaxone: 90%, and aztreonam: 95%.

3.5. Screening and confirmation of ESBLs' production for *E. coli*

Presumptive ESBL-producing *E. Coli* were detected in (17) clinical specimens, were initially screened for ESBLs and result 17(100%) . Of this, 10(58.8%) isolate was determined as ESBLs-producer, following confirmatory testing to be ESBLs producer by (modified double-disc synergy test). Resistance of *E. Coli* to cephalosporins (third generation) and monobactams were as the following: cefotaxime 100%, ceftazidime 100%, ceftriaxone 100%, and aztreonam 100%.

3.6. Screening and confirmation of ESBLs' production for *Proteus mirabilis*

The five *proteus mirabilis* clinical isolates were investigated phenotypically (screen and confirmatory tests) for ESBL production, were initially screened for ESBLs and result 5(100%) The result of the confirmatory examination was 2 (40%). Resistance of *proteus mirabilis* to third generation cephalosporins and monobactams were as the following: cefotaxime: 80%, ceftazidime: 40%, ceftriaxone: 100%, and aztreonam: 40%.

3.7. Screening and confirmation of ESBLs' production for *Acinetobacter baumannii*

the clinical isolates 2 of Acinetobacter baumannii were investigated phenotypically (screen and confirmatory tests) for ESBLs production , were initially screened for ESBLs and result was 2(100%)and the confirmatory assay result was (2 100%) . Resistance of Acinetobacter baumannii to third generation cephalosporins and monobactams were as the following: cefotaxime: 100%, ceftazidime: 100%, ceftriaxone: 100%, and aztreonam: 100%.

3.8. Molecular detecting Antibiotic Resistance Genes in gram negative bacteria

The results observed that all the isolates of *Klebsiella pneumonia*e, E. coli, proteus mirabilis, Burkholderia cepacia and Klebsiella oxytoca) were negative for the presence of bla $_{CTX\text{-}M1}$, bla $_{CTX\text{-}M2}$, and bla *CTX-M*9 genes. Molecular detection of CTX -M ESBLs for isolate *Pseudomonas aeruginosa* showed that 1 (8.3%) of the isolate was positive for at least one *CTX-M* ESBLs gene from 12(60%) , the frequency of *CTX-M* ESBLs gene presence among *Pseudomonas aeruginosa* was as follows: bla$_{CTX\text{-}M1}$,1(8.3%); bla $_{CTX\text{-}M2}$, 1(8.3%); bla $_{CTX\text{-}M9}$ 0(0%) ,there was a significant association between the presence of ESBL genes in *Pseudomonas aeruginosa* and site of infection ,our study consistent with this study with in Jimma, Ethiopia [33,34] containing this bla $_{CTX\text{-}M2}$ in the (3%); (8.2%) respectively. The results was consistent with [35] demonstrated the frequencies of this gene bla$_{CTX\text{-}M1}$ (10%) in Erbil.

Detection of CTX -M ESBLs for isolate Acinetobacter baumannii 1(50%) of the isolate was positive for at least one *CTX-M* ESBLs gene, the frequency of *CTX-M* ESBLs gene from 2(100%) presence among Acinetobacter baumannii was as

follows: bla $_{CTX\text{-}M1}$,0(0%); bla $_{CTX\text{-}M2}$, 1(50%); bla $_{CTX\text{-}M9}$ 0(0%) There was a significant association between the presences of ESBL genes .Our study is similar to these studies in in Saudi Arabia [36] carry this gene bla $_{CTX\text{-}M2}$ (81%) and another study by [37] carry this gene bla $_{CTX\text{-}M2}$ (43%).The agarose gel of PCR products was shown in Figure (3), (4).

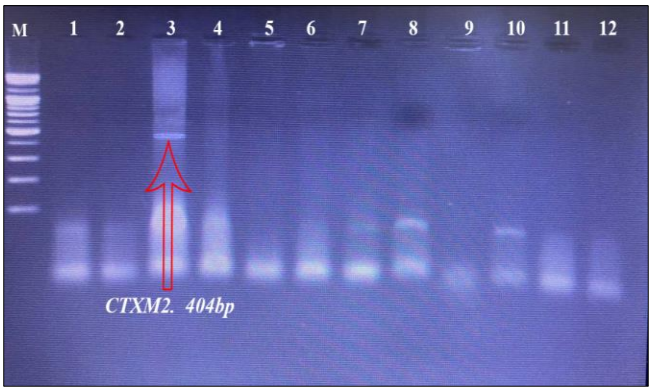


Figure 3 Gel electrophoresis of amplified *CTXM* genes from gram negative bacteria using conventional PCR. Agarose 1.8 %, 100 V/cm for 35 min, visualized on a UV transilluminator. Lane (M): 2000 bp DNA ladder. Lane (1-12 *)* *Acinetobacter baumannii* isolate at product size. *CTXM2* 404 bp.

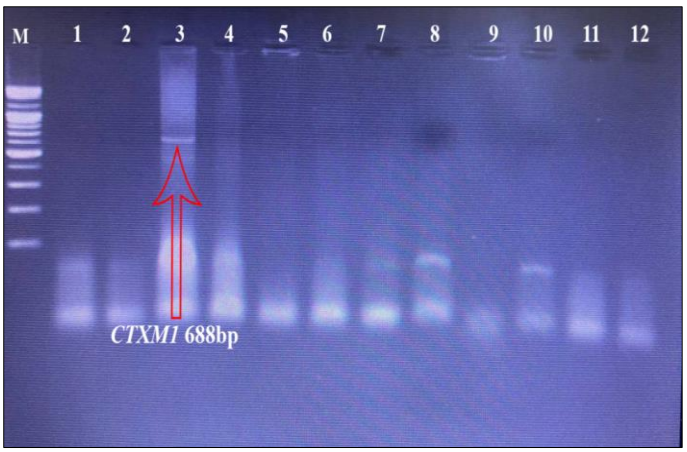


Figure 4 Gel electrophoresis of amplified *CTXM* genes from gram negative bacteria using conventional PCR. Agarose 1.8 %, 100 V/cm for 35 min, visualized on a UV transilluminator. Lane (M): 2000 bp DNA ladder. Lane (1-12): *Pseudomonas aeruginosa isolate at product size.* CTXM 688 1bp

Beta-lactam antibiotics are being used less and less due to their extensive usage, which has led to a rise in the global dissemination of ESBL enzymes. Therefore, the most effective way to preserve the effectiveness of beta-lactam antibiotics is to fully identify ESBL through trials and restrict the consumption of beta-lactam antibiotics [38].

3.9. TEM, SHA, OXA ESBLs

The results were of *bla*$_{SHV}$ 3(23%) from isolates *Klebsiella pneumoniae* .Our study is consistent with these studies [39,40] in US hospitals ,these studies have this gene *bla*$_{SHV}$ (23.5%); (26%) respectively . Compatible with in Wasit [16] this have the gene *bla*$_{SHV}$ (50%). The result of *bla*$_{SHV}$ 4(33%) for *Pseudomonas aeruginosa* ,this result of our study is in agreement with these studies in a Nigerian Teaching Hospital [41,42] that contained this gene in these studies (51%) ; (43%) respectively. The result *bla*$_{SHV}$ of our study was 2(100%) in *Acinetobacter baumannii*,these results of our study are similar to the results of these studies in Mashhad, Iran [43,44] who carry this gene *bla*$_{SHV}$ (100%) ; (99%) respectively.

As for the results of *bla*$_{SHV}$ 1(10%) for one *E. coli* , the results of our study are similar to those of these studies [43,45] in China's Sichuan-Chongqing Circle. who carry this gene *bla*$_{SHV}$ (12%) ; (18%) respectively , and agree with this in Wasit

[46] whose result is 13(29%). With regard to the results *bla*$_{SHV}$ 1(50%) for *proteus mirabilis*. Our results are similar to those of these studies in Japan [47,48] in Poland who carry this gene *bla*$_{SHV}$ (50%) ; (58%) respectively.

The results of the gene *bla*$_{OXA}$ 1(10%) were for *E. coli*, our study of this gene is consistent with the results of these studies [49,50] in Germany that contain this gene *bla*$_{OXA}$ (7%) ; (14.9%) respectively. Correspond to the study in Wasit [14] whose result is (17.8%).As for the result *bla*$_{OXA}$ 1(100%), it was for *Burkholderia cepacia* . Our study is similar to the results of these studies [51,52] in the United States who carry this gene *bla*$_{OXA}$ (93%) ; (100%) respectively .As shown figure (5) .However, TEM gene any positive results in all isolation bacteria in this study did not find.

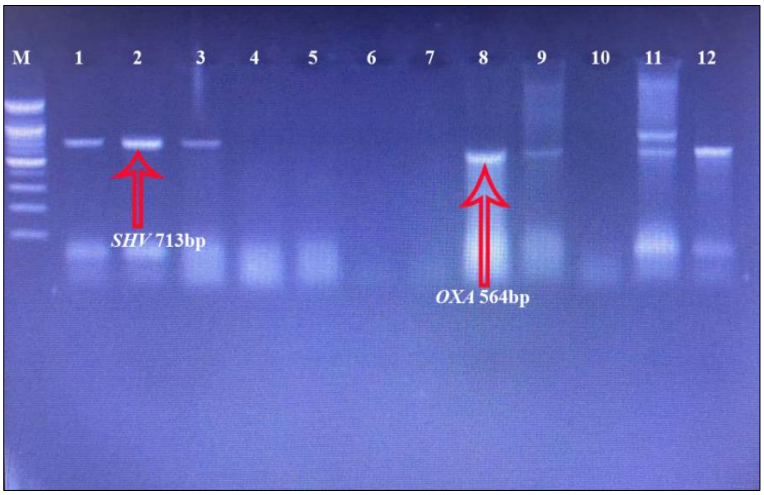


Figure 5 Gel electrophoresis of amplified ESBL genes using conventional PCR. Agarose 1.5 %, 100 V/cm for 40 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 2000 bp DNA ladder. Lane (1-12): some *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Acinetobacter baumannii, E.coli, proteus mirabilis, Burkholderia cepacia* isolate at gene product size. *SHV* 713 bp and *OXA-1*-like 564bp

4. Conclusion

According to the findings of current study in ICU, The highs frequency of specimens collection from intensive care unit patients were isolated from sputum and the gram-negative bacteria were the most common than the positive bacteria. Results of antibiotic susceptibility test revealed that the most active compound against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were Amikacin. Phenotypically and genotypically of ESBLs for *klebsiella pneumoniae* showed no isolate had ESBLs producers *CTX-M*-type ESBL. The most prevalence of *bla*$_{SHV}$ and no isolate have *bla*$_{TEM}$ and *bla*$_{OXA-1}$ genes, Phenotype and genotype of *pseudomonas aeruginosa* ESBL producers were carrying *CTX-M*2 and *CTX-M*1 in their isolates, also prevalence of *bla*$_{SHV,}$ Amp$_{C\beta}$- lactamase comprised (*bla*$_{EBC)}$, Phenotype and genotype of ESBLs for *E.Coli* showed no isolate had ESBLs producers *CTX-M*-type ESBL. Also carries *bla*$_{SHV,}$ *bla*$_{OXA}$-1 and no isolate have bla$_{TEM}$ gene.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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