

RESEARCH ARTICLE

Prevalence and Antibiotic Susceptibility among Gram Negative Bacteria Isolated from Intensive Care Units at a Tertiary Care Hospital in Riyadh, Saudi Arabia

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Abstract

Antibiotic resistance is an essential issue mostly in the intensive care units (ICUs). The Goal of this study was to investigate the widespread of multidrug resistance (MDR) gram-negative bacterial pathogens isolated from ICUs at King Khalid University Hospital (KKUH), Riyadh, KSA, and their ability to produce ESBL and MBL enzymes. All organisms were isolated from different ICUs at (KKUH) between June to December 2016. Identification and antimicrobial susceptibility were committed according to the laboratory policy. The bacterial Isolates flagged as ESBL or MBL by Vitek 2 were confirmed using E-test method recommended by CLSI. 70 isolates from different body sites comprising 25 (35.7 %) were *P. aeruginosa*, 23 (32.9 %) were *K. pneumoniae*, 16 (22.9%) were *E. coli*, and 6 (8.6%) were *A. baumannii*. Among the 23 isolates *K. pneumoniae* and 16 of *E. coli*, 19 (82.6%) and 16 (100%) were detected as ESBL (+) by double-disk diffusion method according to guidelines of CLSI. On the contrary, ESBL was not detected in any isolates of *P. aeruginosa* or in *A. baumannii*. All *P. aeruginosa* and *A. baumannii* isolates were carbapenem resistant. MBL was found in all *P. aeruginosa*, *A. baumannii* and 4 (17.4 %) of *K. pneumoniae* where *E. coli* strains did not appear any MBL action. The essential resistance mechanisms in the evaluated strains were ESBL and MBL. Molecular testing is recommended to confirm the phenotypic results and to detect the resistant genes.

Keywords: MDR gram negative bacteria, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *A. baumannii*, ESBL, MBL.

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INTRODUCTION

The growth of MDR gram negative bacteria is an important healthcare challenge worldwide¹. The infections by MDR gram negative pathogens often lead to enhanced mortality, prolonged hospital stays and unreasonable healthcare costs. In total, this evidence emphasize the significance for hospitals to start reacting in a proactive way rather than in a reactive one to withstand the increasing resistance rates².

The increase at intervals percentages of antibiotic resistance can be a serious interest in every non-fermenting bacilli and isolates of the Enterobacteriaceae family. β -lactams are the base of medication for genuine contaminations. The Most effective of those are the carbapenems, which are recommended to be used for the treatment of infections brought about by strains of the emerging Enterobacteriaceae, principally *Escherichia coli* and *Klebsiella pneumonia*, and non-fermenters, *Pseudomonas* spp., and *Acinetobacter* spp³, which possess extended-spectrum- β -lactamase (ESBL).

β -lactamases are proteins made by specific bacteria that break down the β -lactam ring of β -lactam anti-infection agents (monobactams, penicillin's, cephalosporins, carbapenems, and monobactams), is one amongst the foremost necessary mechanisms of microbial resistance to β -lactam antibiotics⁴. The decomposed β -lactam drugs result in a dormant product once the ring is broken⁵.

One of the foremost crucial resistant mechanisms in Gram-negative bacteria against β -lactam anti-infection agents is iatrogenic by the creation of β -lactamase catalysts. β -lactamase proteins could be characterized into four gatherings which are: A, B, C and D. Predictable with this order, expansive range β -lactamases are sorted among gathering A. The Gram-negative microscopic organisms have brisk enlarged protection from expansive range β -lactam antimicrobial agents. In excess of 200 kinds of expanded range β -lactamases (ESBLs) are discovered around the world, most having a place with the Enterobacteriaceae family⁶.

Resistance in Gram-negative microscopic organisms is principally mediated through the yield of (ESBL), AmpC β - lactamases and Carbapenemases⁷.

Extended-spectrum β -lactamases (ESBLs) are bacterial enzymes that hydrolyze β -lactam anti-infection agents, such as, penicillins, cephalosporins and monobactams. They represent the foremost mechanism of resistance to second, third, and fourth generation cephalo-sporins, for example, cefuroxime, cefotaxime, ceftriaxone and ceftazidime⁸.

Carbapenemases are β - lactamases that exemplify Metallo- β -lactamases (MBL) and serine- β -lactamases (KPC, GES, OXA, etc). Conversely, throughout the past few years, Carbapenem resistance has been progressively recorded among Enterobacteriaceae and is predominantly recognized by the production of Ambler class B procured Metallo- β -lactamases (MBLs)⁹.

MBLs producing Gram-negative bacteria is an emerging threat worldwide ascribed to their resistance to any or all β -lactams except aztreonam¹⁰. The genes of MBL are either transferable plasmids or a piece of the bacterial chromosome¹¹.

Aim of the work

To detect the antimicrobial resistance pattern, ESBL and MBLase producers among strains of gram-negative bacteria were secluded from King Khalid University Hospital ICUs.

MATERIAL AND METHOD

Bacterial isolates

This retrospective study was done at King Khalid University Hospital, Riyadh, Saudi Arabia. The study included 70 successive, non-copied clinical isolates of ESBL *K. pneumoniae*, *E. coli*, MDR *P. aeruginosa* and *A. baumannii* that were identified from June to December 2016. These isolates were from various specimen types including, blood, wound, sputum, tracheal aspirate, eye, and groin, which were routinely cultured in the bacteriology laboratory.

Identification of the organisms and susceptibility testing

Identification and antimicrobial susceptibility of 70 isolates belonging to different species of gram negative microscopic organisms were carried out according to the laboratory policy by Vitek 2 (Biomerieux, Marcy l'Etoile). As indicated by the instructions of the Clinical Laboratory Standard Institute¹² (CLSI), antibiotic susceptibilities were confirmed by Kirby-Bauer

disk diffusion method. The selected strains of gram negative bacteria were tested for a group of antibiotics such as ceftazidime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), levo-floxacin (5µg), gentamicin (10µg), imipenem (10µg), meropenem (10µg), piperacillin- tazobactam (100/10µg), ceftiofloxacin (30µg), cefuroxime (30µg), amoxicillin/ clavulanic acid (20/10µg), aztreonam (30µg) and amikacin (30µg), tigecycline (15µg), and colistin (10µg), (Oxoid, England).

Phenotypic detection of ESBLs and MBLs

All strains were examined for ESBL production by CLSI phenotypic corroborative test by double-disk diffusion method¹². ESBL generation was affirmed by E-test ESBL strips (AB Biodisk, Solana, Sweden).

All strains were investigated for Metallo-beta-lactamase production by utilizing the Etest MBL strip (AB Biodisk, Solana, Sweden), containing two-sided seven-dilution scope of imipenem(IP) and imipenem+ EDTA (IPI). The test was done with accordance to the manufacturer’s directions MIC ratio of ≥8 for the two reagent sides, a phantom zone between IP/IP inhibitor and distortion of each ellipse was considered as a positive result¹³.

Statistical analysis

All data were stored in Microsoft Excel, Version 2016. Data management and statistical analyses were also performed in Excel. Descriptive statistics of the data and variables are shown in the form of frequencies and percentages.

ETHICAL STATEMENT

The Institutional Review Board, college of medicine, King Khalid University Hospital, Riyadh, Saudi Arabia, has granted permission to conduct this study.

RESULTS

Bacterial isolates

The study included 70 isolates from different body sites comprising 25 (35.7%) were *P. aeruginosa*, 23 (32.9 %) were *K. pneumoniae*, 16 (22.9 %) were *E. coli*, and 6(8.6 %) were *A. baumannii*, are shown in table 1.

The samples were secluded from numerous clinical specimens such as tracheal aspirate(n=23; 33%), sputum (n= 13 ;19%),urine (n= 11 ;16%), blood (n=10 ; 14%), wound (n=5 ;7%), eye (n=3; 4%), body fluid (n= 2; 3%), genital, groin and tissue (n=3;3%), mainly from ICUs (SICU,

Table 1. Frequencies and percentages of various Gram-negative bacteria isolated from ICUS (n = 70)

Organisms	Frequency (N=70)	Percentage
<i>Pseudomonas aeruginosa</i>	25	35.7 %
<i>Klebsiella pneumoniae</i>	23	32.9%
<i>Escherichia coli</i>	16	22.9%
<i>Acinetobacter baumannii</i>	6	8.6 %

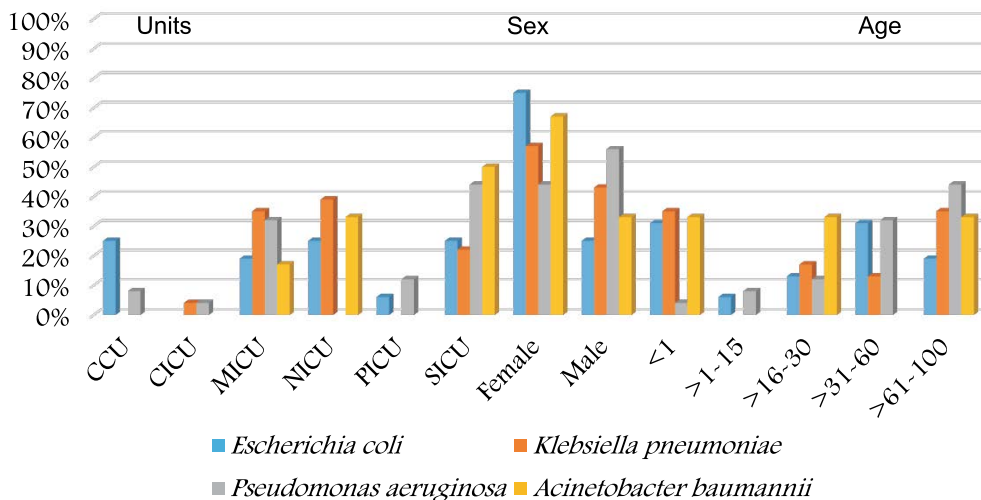


Fig. 1. Demographic characteristics of the patient populations.

n=23 ; 33%, MICU, n=20; 29%, NICU, n=15; 21% , CCU, n= 6; 9%, PICU, n= 4;6% , CICU, n=2;3%. From different age groups, (>61-100 ,n/N: 24/70: 34%, >31-60, n/N: 16/70: 23%,<1, n/N: 15/70: 21%, >16-30, n/N: 11/70: 16%, >1-15, n/N: 4/70: 6 % . Forty isolates which represents (57%) were from female and thirty isolates (43%) from male. The demographic characteristics of the patient populations are shown in (Fig. 1).

Antibiotic susceptibility testing

The antibiotic resistance pattern of *K. pneumoniae* and *E. coli* is shown in (Fig. 2). The isolates of *K. pneumoniae* demonstrated a high percentage of resistance to Ceftazidime 22 (96%), followed by cefotaxime and cefuroxime 20 (87%), ciprofloxacin 9 (39%), amoxicillin/clavulanic, gentamicin 8 (35 %). The strains revealed a low percentage of resistance to meropenem, Imipenem 2 (8.6%) and to amikacin 1 (4%). Also for *E.coli* displayed a high percentage of resistance to Ceftazidime, cefotaxime and cefuroxime 9 (56%) also followed by Ciprofloxacin 7 (44%), levofloxacin and gentamicin 6 (38%), amoxicillin/clavulanic 4 (25%). The strains revealed a low percentage of resistance to Ceftriaxone 1(6%).

As for *P. aeruginosa* and *A. baumannii*,

the antibiotic resistance pattern is shown in (Figure 3). The isolates of the *P. aeruginosa* revealed a high percentage of resistance to imipenem and meropenem 21(84%) and 12 (48%), followed by ceftazidime 10(40%), levofloxacin 8 (32%), ciprofloxacin and pipe-racillin/tazobactam showed the same percentage of resistance 7(28%), followed by amikacin 1 (4%). The strains revealed a low percentage of resistance to gentamicin 4(16%). Also, the *A. baumannii* strains revealed a high percentage of resistance 5(83%) for imipenem, meropenem, ciprofloxacin, ceftazidime, and gentamicin, followed by amikacin 4(67%) followed by Tigecycline and colistin 2 (33%).

Phenotypic detection of ESBLs and MBLs

Among the 23 isolates *K. pneumoniae* and 16 of *E. coli* ,19 (82.6%) and 16 (100%) were ESBL producers, sequentially. As opposed to this outcome, ESBL was not detected in any strains of *P. aeruginosa* or in *A. baumannii*.

All *P. aeruginosa* and *A. baumannii* strains were carbapenem-resistant. MBL was detected in all the specimens of *P. aeruginosa*, *A. baumannii* and 4 (17.4%) of *K. pneumoniae* where *E coli* specimens did not demonstrate any MBL action (Table 2).

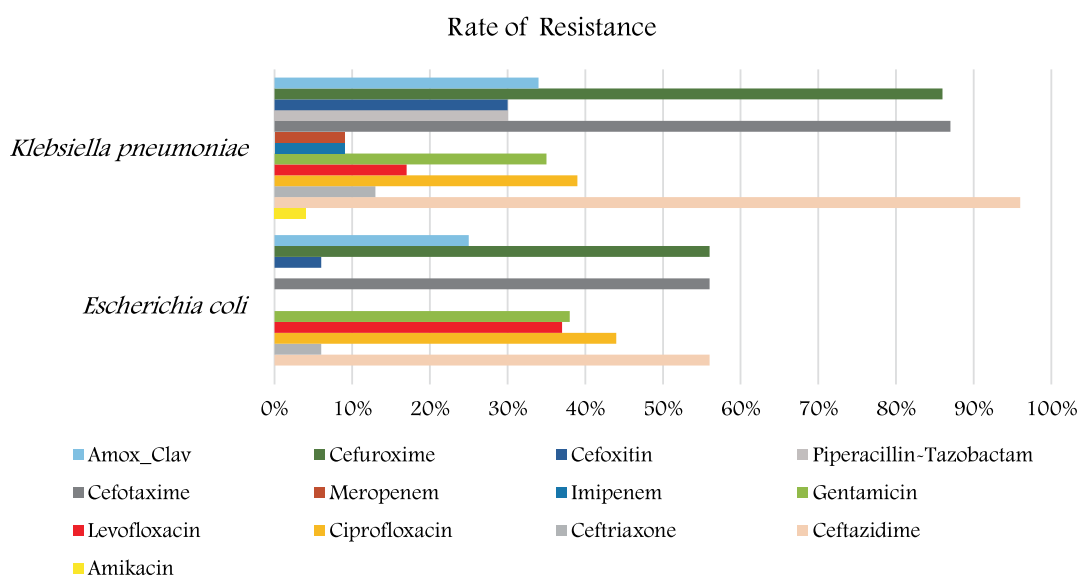


Fig. 2.The antibiotic resistance pattern of *K. pneumoniae* and *E. coli*.

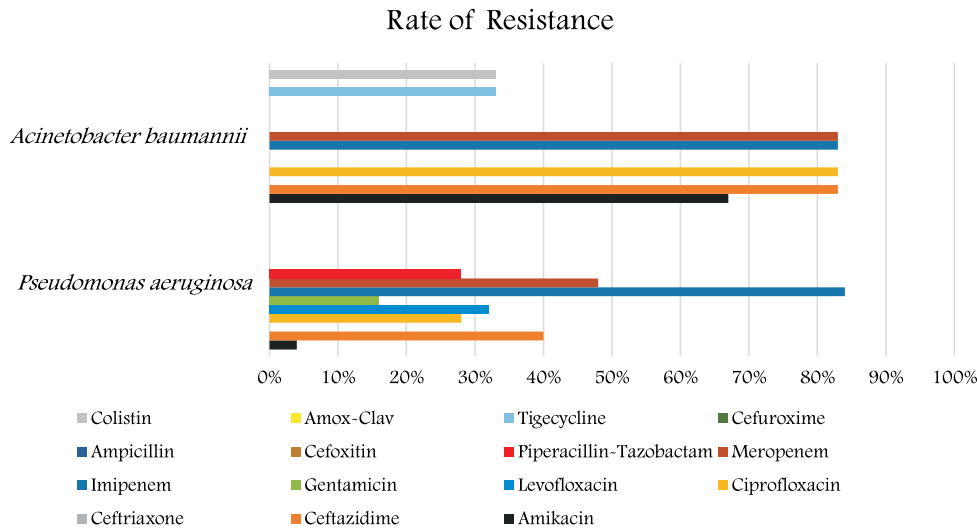


Fig. 3. The antibiotic resistance pattern of the *P. aeruginosa* and *A. baumannii*.

Table 2. A number of ESBL and MBL producing bacteria detected by phenotypic tests.

Organism	ESBL (N = 35)	MBL N = 35
<i>Acinetobacter baumannii</i>	0	6
<i>Klebsiella pneumoniae</i>	19	4
<i>Escherichia coli</i>	16	0
<i>Pseudomonas aeruginosa</i>	0	25

DISCUSSION

There is a remarkable increase of multidrug-resistant (MDR) gram-negative bacteria in hospitals and significantly in the intensive care unit (ICU) setting. The clinical outcomes of infections brought about by MDR pathogens as yet dubious¹⁴. The resistance pattern is most likely noted in ICUs due to the unlimited use of antibiotics in these units compared to the other hospital departments¹⁵ and 70% of these infections were caused by GNB¹⁶.

In this study, most of bacterial pathogens were recovered from female patients. This may be due to higher antibiotic intake prior to hospitalization in women¹⁷. In contrast with Ibrahim’s findings (2018), who stated that the majority of bacterial pathogens were recovered from male patients¹⁸. Moreover, most of the isolates were recovered from the respiratory system and this finding agrees with a previous

surveillance studies in Riyadh indicated that the most of bacterial strains were from the respiratory tract^{19, 20}. Our findings indicated that respiratory diseases were the most widely recognized in our ICUs.

In the current study, the most commonly isolated organism was *P. aeruginosa* followed by *K. pneumoniae*, *E. coli*, and *A. baumannii*.

In a study of Altun et al., (2013), who studied 78 specimens isolated from ICUs, Altun et al., (2013) found that *A. baumannii* was the most normally isolated organism pursued by *Klebsiella* spp., *E. coli*, and *P. aeruginosa*²¹.

According to Al Johani et al., (2010), who found that *Acinetobacter baumannii* was the most frequently organism, pursued by *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, and *Enterobacter*²². Also, Ibrahim (2018) reported that the most commonly secluded pathogens were *Acinetobacter* spp. (27.2%) followed by *P. aeruginosa* (23.8%) and *K. pneumoniae* (18.6%)¹⁸.

Here in our study, the isolates of *K. pneumoniae* demonstrated a high percentage of resistance to Ceftazidime (96%), followed by cefotaxime and cefuroxime (87%), ciprofloxacin (39%), amoxicillin/clavulanic and gentamicin (35 %). Ibrahim (2018) reported that more than 60% of *K. pneumoniae* strains were resistant to cephalosporins, trimethoprim/sulfamethoxazole, aztreonam, piperacillin, and piperacillin/

tazobactam. The resistance rates for aminoglycosides, carbapenems, and cipro-floxacin were between 40% and 55%. In another setting in Saudi Arabia, *K. pneumoniae* demonstrated the highest resistance rates for ceftriaxone (59.4%), aztreonam (58.3%), and ceftazidime (58.3%)¹⁸.

Results of the present study reported that *E. coli* specimens demonstrated a high percentage of resistance to Ceftazidime, cefotaxime, and cefuroxime (56%). Amer et al., (2017), stated that most of *E. coli* isolates exhibited ESBL activity, and resistance is significantly increased in all beta-lactams antibiotics especially ceftazidime (38% to 100%); while the previous surveillance study showed *E. coli* resistance to ceftazidime (24% to 54%)²³.

In our study, *P. aeruginosa* showed a high percentage of resistance to imipenem and meropenem (84%) and (48%). Where Emeka et al., (2018), reported that resistance percentages for *P. aeruginosa* were as per the following: Augmentin (47.4%), Cefuroxime (52.6%), Ceftriaxone (100%)²⁴.

In the present investigation, *A. baumannii* isolates showed a high percentage of resistance (83%) for imipenem and meropenem which is similar to the finding of Ibrahim (2018), who reported that high resistance rates for carbapenems such as imipenem (97.5%) and meropenem (96.2%)¹⁸. The high resistant rates pose a major concern in our ICU as carbapenems are the medication of decision for *Acinetobacter* infections. Our findings suggest the existence of carbapenemase-producing strains, which has also been reported in other studies in Saudi Arabia and Gulf Cooperation Council States^{25,26}. Therefore, determining the carbapenem-resistance mechanisms could lead to improvements in the outcomes of infections brought about by these microscopic organisms²⁵.

In the present investigation, ESBL was detected in 35 of 39 (90%) isolates (19 of *K. pneumoniae* and 16 of *E. coli*). ESBL was distinguished by Vitek 2 compact system.

In the investigation of Altun et al.²¹, ESBL was affirmed in 19 of 27 (70%) of the bacterial specimens (12 *E. coli* and 19 *K. pneumoniae*), ESBL was distinguished by Vitek 2 compact system. In the investigation of Baykal et al., ESBL was affirmed by combined disc method in 26 *E. coli* and 70 *K. pneumoniae* strains that were examined²⁷.

In the current investigation, MBL was affirmed in all the bacterial specimens of *P. aeruginosa* and *A. baumannii* 31 of 35 (86%). *P. aeruginosa* displayed resistance to anti-infection agents as a consequence of a permeability barrier of the cell surface, multidrug efflux pumps, and yielding of β -lactamases (AmpC β -lactamase, ESBLs, and MBLs)²⁸. MBL generation in *P. aeruginosa* in the current investigation (71%) is comparable with Altun et al.²¹ (57%) and Tankhiwale (16.87%)²⁹.

Carbapenemase-generation *Acinetobacter* strains are rising worldwide due to frequent carbapene utilization and the choice of resistant bacteria under antibiotic pressure³⁰⁻³².

In our study, it was found that 6 of 6 (100%) *A. baumannii* strains were MBL producers by E test. Where Altun et al. (2013) stated that 31 of 44 (70%) *A. baumannii* strains were detected as MBL producers²¹.

CONCLUSION

To sum up, Gram-negative bacterial resistance is still a major issue in KKHU ICUs, Riyadh. ESBL and MBL were the fundamental resistance patterns of the strains assessed in this investigation. The most commonly secluded organisms were *P. aeruginosa* pursued by *K. pneumoniae*, *E. coli*, and *A. baumannii*. ESBL was confirmed in 35 of 39 (90%) isolates (19 of *K. pneumoniae* and 16 of *E. coli*). And MBL was affirmed in all the specimens of *P. aeruginosa* and *A. baumannii* 31 of 35 (86%). Imipenem, Meropenem, and Amikacin were the most effective agents for *K. pneumoniae* and *E. coli*. Colistin was the drug of choice for most cases of *A. baumannii*. *P. aeruginosa*, Amikacin and Colistin were the most effective agents.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

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