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*Article*

# Associated Risk Factors and Pathogen Burden among ICU Patients in a Tertiary Care Hospital in Hail Saudi Arabia with Particular Reference to β-Lactamases Profile

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**Abstract:** A 1-year prospective study was carried out on patients in the ICU unit at a tertiary care hospital, Hail, Kingdom of Saudi Arabia. A total of 163 bacterial isolates were obtained from different clinical specimens with a high proportion of bacteria found associated with ventilator-associated pneumoniae (70, 43%), followed by catheter-associated urinary tract infection (39, 24%), central line-associated bloodstream infection (25, 15%), and surgical site infection (14, 8.6%). *Klebsiella pneumoniae* was the most common isolate (39, 24%), followed by *Acinetobacter baumannii* (35, 21.47%), *Pseudomonas aeruginosa* (25, 15%), and *Proteus spp* (23, 14%). Among the highly prevalent bacterial isolates, extended-spectrum beta-lactamase was predominant (42, 42.4%). Proper use of antibiotics, continuous monitoring of drug sensitivity patterns, and taking all precautionary measures to prevent beta-lactamases-producing organisms in the clinical settings are crucial and significant factors to fend off life-threatening infections and for a better outcome.

**Keywords:** ICU; VAP; ESBL; Klebsiella pneumoniae; Acinetobacter baumannii

## 1. Introduction

Artificial ventilation clinches the proper maintenance of gas exchange vital for the body, which is considered therapeutic support for patients with respiratory and metabolic disturbance in the intensive care unit (ICU). However, it divulges the compromised patients towards acquiring ventilator-associated pneumonia (VAP). It is perceived that intubation in ICU patients acts as a trigger for VAP by forming biofilm on its surface, subscribing to the pathogenesis of the infection [1]. Also, it plays a vital role in deteriorating the effectiveness of antimicrobial agents as biofilm act as a diffusional barrier against the antibiotics making those less effective, stretching the morbidity and mortality rates [2].

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In all mechanically ventilated patients, the chance of developing VAP ranges from 9-27 %, with the highest risk being early during hospitalization [3]. VAP rates range from 1.2 to 8.5 per 1,000 ventilator days and rely on the definition used for diagnosis. During the first five days of mechanical ventilation, the risk is 3%, with 3.3 days of the mean duration. However, the risk factor dropped to 2% per day between days 5 to 10 of ventilation and 1% per day after that [4]. Mainly, the community-originated infectious agents are responsible for early-onset of VAP. These comprise pathogens like Methicillin-resistant *Staphylococcus aureus* (MRSA), *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, or *Enterobacteria* susceptible to antimicrobials. And the occurrence of opportunistic pathogens is seen with late-onset VAP, such as *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and another *Acinetobacter spp*., [5].

The early diagnosis of VAP is quite complex. Signs and symptoms, chest radiography, and diagnostic tests are the main parameters for VAP diagnosis. Still, there is no gold-standard method for diagnosing pneumonia, and the claim used to define this has the least sensitivity and specificity to establish such a diagnosis [6]. Hence the support provided by microbiological data to refine the diagnostic accuracy is very important [7]. The present study identified and characterized clinical isolates while considering their antibiotic susceptibility.

## 2. Materials and Methods

### Sample area

A prospective study over one year, from January 2019 to December 2019, was conducted at King Khalid hospital, Hail, Kingdom of Saudi Arabia (KSA). The study population comprised 591 clinically suspected hospital-associated infections (HAI) cases. The study protocol was approved by the Ethics Committee, Research Deanship, University of Hail, Saudi Arabia. Informed consent was obtained from the patients before the start of the study.

### Clinical sampling

Respiratory secretions were collected in a screw-capped sterile universal container with a wide mouth for early morning sputum samples, endotracheal aspirates, and bronchial aspirates. 10ml of venous blood was collected from adult patients following strict aseptic precautions. 10ml of urine sample was collected from the catheter tube using a sterile disposable syringe in a sterile universal container from each patient. The site of aspiration in the catheter tube was primarily cleaned using 70% ethanol, and then the catheter tube was clamped proximally to the urethral or suprapubic opening to allow for the collection of freshly voided urine.

Pus samples were collected from the infected wound using a sterile disposable syringe. If the sample amount was inadequate, the sample was collected by using a transportable cotton swab by rolling in all directions with sufficient pressure to get any amount of exudate if possible. Samples were collected using a sterile cotton swab rolled in all directions.

Fluid samples like pleural fluid were collected using a sterile syringe under aseptic conditions. Specimens were collected in anticoagulants containing tubes to avoid clotting [8].

### Phenotypic identification

Standard conventional biochemical and microbiological tests were performed for phenotypic identification of the isolates. Further confirmation was done using the BD Phoenix M50 system (BD Diagnostic Systems, Oxford, UK), in which identification was based on conventional, chromogenic, and fluorogenic reactions.

BD Phoenix M50 test procedure: Gram staining was done to select the appropriate Phoenix panel for inoculation. Colonies of the same morphology were picked with the tip of a sterile cotton swab from the growth on the appropriate solid media and suspended in the Phoenix ID broth (4.5ml). A nephelometer was used to adjust the turbidity of the

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inoculum to 0.5-0.6 of the McFarland turbidity standards. The ID tube inoculum was poured to fill the port on the ID sides. The panels were sealed, logged, loaded into the Phoenix, and incubated at 35oC. Data were automatically generated and collected by the system every 20 minutes. After 16 hrs of incubation, the Epicenter data management software version 6.61A (BD Diagnostic Systems) was used to analyse the result.

**Antibiotic susceptibility testing**

Kirby-Bauer disk diffusion method was used to test the susceptibility of the isolates against various antibiotics following Clinical and laboratory standards institute guidelines 2020 [9]. The antibiotics used included cefoxitin (30µg), ceftazidime (30µg), cefotaxime (30µg), cefpodoxime (30µg), aztreonam (30µg), cefepime (30µg), imipenem (10µg), amikacin (30µg), ciprofloxacin (30µg), gentamicin (10µg), ampicillin/sulbactam (20/10µg), piperacillin/ tazobactam (100/10µg), tigecycline (15µg) and colistin (10µg) discs. The diameters of the zones of inhibition were recorded and interpreted as sensitive and resistant, according to the CLSI guidelines 2020 [9], except for colistin and tigecycline, which are not in CLSI guidelines for Acinetobacter species. Keeping the breakpoints of ≤2 as sensitive and ≥4 as resistant [9], the zone sizes of colistin were taken as ≥11mm susceptible and ≤10mm resistant [10]. According to Jones et al., the interpretation for tigecycline was ≥16 mm as sensitive and ≤12 as resistant [11].

**β-Lactamases profiling of clinical isolates:**

Common steps used before performing phenotypic methods

a) Few bacterial colonies were picked by the inoculating loop and mixed in nutrient broth, incubated at 37oC for 15-20 minutes. Then the turbidity of the broth was matched with 0.5 McFarland standards.

b) Excess inoculum was drained by squeezing the swab on the mouth of the tube.

c) Carpet culture was done on Mueller Hinton Agar (MHA) plate with a sterile cotton swab with a regular rotation of the swab stick for uniform distribution.

d) The inoculated plate was allowed to dry for 5-10 minutes before placing the antibiotic disks.

**Detection of antibiotic resistance:**

ESBL detection:

For ESBL detection, a disk of ceftazidime (30µg) and ceftazidime+clavulanic acid (30/10µg) was incorporated on MHA plates and incubated at 37oC for 16-18 hrs. A ≥5mm increase in zone diameter for either antimicrobial agent is considered a positive beta-lactamase enzyme.

AmpC detection:

Clinical isolates which come up with a synergistic effect with Cefepime only in a modified double-disk synergy test (MDDST) were further tested for the AmpC enzyme production by AmpC disk test after an initial screening with a Cefoxitin (30 µg) disk.

Determination of carbapenemase production:

Isolates resistant to one or more carbapenems, i.e., imipenem and/or meropenem, were subjected to the Modified Hodge test (MHT) as described earlier [9].

## 3. Results

A total of 163 bacterial isolates were identified and isolated from the different clinical samples during the study. As shown in Table-1, the prevalence of VAP was the highest (70, 43%), followed by catheter-associated urinary tract infection (CAUTI; 39, 24%), central line-associated bloodstream infection (CLABSI; 25, 15%), surgical site infection (SSI; 14, 8.6%), non-surgical site infection (non-SSI; 7, 4.3%), bloodstream infection (BSI; 5, 3%), and the lowest prevalence was for respiratory tract infection (RTI; 3, 1.8%). In the case of VAP, the most predominant pathogen reported was *Acinetobacter baumannii* (18, 51%), while *Klebsiella pneumoniae* was the predominant pathogen for CAUTI (11, 28%) and CLABSI cases (6, 24%). As shown in Table-1, *A. baumannii* was the most common pathogen isolated from surgical site infection cases (9, 64%).

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**Table-1:** Pathogen burden among different types of infections in ICU patients.

| No | Pathogen | VAP | CAUTI | CLABSI | SSI | Non-SSI | BSI | RTI | Total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Pseudomonas aeruginosa* | 9 (12.8%) | 9 (23%) | 4 (16%) | 2 (14.2%) | 1 (14.2%) | -- | -- | 25 |
| 2 | *Acinetobacter baumannii* | 18 (25.7%) | 2 (5.1%) | 4 (16%) | 9 (64.2%) | 1 (14.2%) | 1 (20%) | -- | 35 |
| 3 | *Enterobacter spp.* | 4 (5.7%) | -- | -- | 1 (7.1%) | | -- | -- | 5 |
| 4 | *Klebsiella pneumoniae.* | 15 (21.4%) | 11 (28.2%) | 6 (24%) | 1 (7.1%) | 3 (42.8%) | 1 (20%) | 2 (66.6%) | 39 |
| 5 | *Staphylococcus epidermidis* | -- | -- | 1 (4%) | -- | | -- | -- | 1 |
| 6 | *Proteus spp.* | 14 (20%) | 6 (15.3%) | 2 (8%) | -- | | 1 (20%) | -- | 23 |
| 7 | *Providencia stuartii* | 3 (4.2%) | 3 (7.6%) | 2 (8%) | 1 (7.1%) | 1 (14.2%) | 1 (20%) | 1 (33.3%) | 12 |
| 8 | *Moraxella catarrhalis* | 1 (1.4%) | -- | -- | -- | | -- | -- | 1 |
| 9 | *Serratia marcescens* | 2 (2.8%) | -- | 1 (4%) | -- | | -- | -- | 3 |
| 10 | *Burkholderia cepacia* | 1 (1.4%) | -- | -- | -- | | -- | -- | 1 |
| 11 | *Morganella morgine* | -- | -- | 1 (4%) | -- | | -- | -- | 1 |
| 12 | *MRSA** | 1 (1.4%) | -- | 4 (16%) | -- | | 1 (20%) | -- | 6 |
| 13 | *Xanthomonas malatophila* | 1 (1.4%) | -- | -- | -- | | -- | -- | 1 |
| 14 | *Escherichia coli* | 1 (1.4%) | 8 (20.5%) | -- | -- | 1 (14.2%) | -- | -- | 10 |
| | **Total** | **70** | **39** | **25** | **14** | **7** | **5** | **3** | **163** |

Table-2 shows the distribution of pathogens associated with ICU infections. *K. pneumoniae* was the predominant (39, 24%), followed by *A. baumannii* (35, 21%), *P. aeruginosa* (25, 15%), and *Proteus spp.* (23, 14%). Other bacterial pathogens were also isolated from the clinical samples, but their prevalence rate was low, as shown in Table-2.

**Table-2:** Prevalence of different bacterial isolates among ICU patients.

| S. No. | Pathogen | Prevalence (%) |
|---|---|---|
| 1 | *Pseudomonas aeruginosa* | 25 (15.33%) |
| 2 | *Acinetobacter baumannii* | 35 (21.47%) |

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| | | |
|---|---|---|
| 3 | *Enterobacter spp.* | 05 (3.06%) |
| 4 | *Klebsiella pneumoniae.* | 39 (23.92%) |
| 5 | *Staph epidermidis* | 01 (0.61%) |
| 6 | *Proteus spp.* | 23 (14.11%) |
| 7 | *Providencia stuartii* | 12 (7.36%) |
| 8 | *Moraxella catarrhalis* | 01 (0.61%) |
| 9 | *Serratia marcescens* | 03 (1.84%) |
| 10 | *Burkholderia cepacia* | 01 (0.61%) |
| 11 | *Morganella morgine* | 01 (0.61%) |
| 12 | *MRSA** | 06 (3.68%) |
| 13 | *Xanthomonas malatophila* | 01 (0.61%) |
| 14 | *Escherichia coli* | 10 (6.13%) |
| | **Total** | **163** |

***MRSA-methicillin resistant *Staphylococcus aureus***

Table-3 shows the month-wise distribution of infections associated with ICU patients. The highest healthcare-associated ICU infections were seen in September (19, 11.7%), followed by the month December and January 17 (10.4%), May (16, 9.8%), April (15, 9.2%), with the least in March (9, 5.5%).

**Table-3:** Month-wise distribution of infections associated with ICU patients

| HCAI | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VAP** | 5 | 5 | 3 | 7 | 4 | 5 | 7 | 6 | 7 | 7 | 4 | 10 | **70** |
| **CAUTI** | 3 | 3 | 3 | 2 | 1 | 5 | 2 | 3 | 6 | 3 | 4 | 4 | **39** |
| **CLABSI** | 2 | 1 | 1 | 2 | 3 | | 3 | 4 | 5 | 1 | 2 | 1 | **25** |
| **SSI** | 5 | 1 | | | 4 | | | | | 1 | 1 | 2 | **14** |
| **Non-SSI** | | | 2 | 1 | 2 | | | | 1 | 1 | | | **7** |
| **BSI** | 2 | | | 1 | 1 | 1 | | | | | | | **5** |
| **RTI** | | | | 2 | 1 | | | | | | | | **3** |
| **Total** | **17** | **10** | **9** | **15** | **16** | **11** | **12** | **13** | **19** | **13** | **11** | **17** | **163** |

Table-4 shows the beta-lactamases profile of the most prevalent gram-negative bacteriae isolated in ICU patients. ESBL production was the highest in K. pneumonia (22, 56%, Figure-1), followed by A. baumannii (12, 34.2%), and the lowest was in P. aeruginosa (8, 32%). In the same manner, AmpC production was highest in K. pneumonia (7, 17.9%), followed by P. aeruginosa (4, 16%) and least for A. baumannii (5, 14.2%). In contrast to ESBL and AmpC, MBL production was not seen in K. pneumoniae, only A. baumannii (8, 22.8%) and P. aeruginosa (6, 24%) isolates were producing MBL. Few bacterial isolates were producing both ESBL and AmpC as shown in Table-4.

**Table-4:** Various beta-lactamases among highly prevalent bacterial isolates

| Types of Beta lactamases | *P. aeruginosa* | *A. baumannii* | *K. pneumoniae* | TOTAL |
|---|---|---|---|---|
| ESBL | 08 (32%) | 12 (34.3%) | 22 (56.4%) | **42** |

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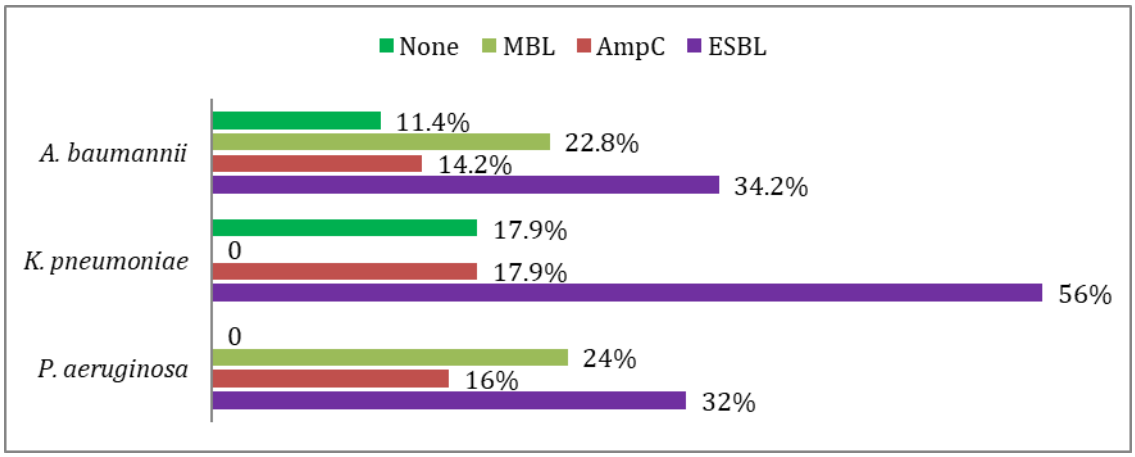
| | | | | |
|---|---|---|---|---|
| AmpC | 04 (16%) | 05 (14.3%) | 07 (17.9%) | **16** |
| MBL | 06 (24%) | 08 (22.9%) | -- | **14** |
| Both (AmpC+MBL) | 07 (28%) | 06 (17.1%) | 03 (7.7%) | **16** |
| None | -- | 4 (11.4%) | 7 (17.9%) | **11** |

**Fig 1:** Graphical representation of beta-lactamases




Our study has shown that endotracheal intubation and mechanical ventilation (p<0.01) were the dominant risk factors significantly found associated with *Pseudomonas* infection in ICU, with chronic obstructive pulmonary disease (COPD) (p=0.001) as the significant clinical type. Prolonged ICU stay that is 1-2 weeks (P=0.025) and >2 weeks (P = 0.0004) were depicted as a significant factor associated with ICU infections.

## 4. Discussion

In our study, we found that the gram-negative bacilli (GNB) infections in ICU patients are extremely high (155/163, 95%), and this may be due to the adoption of inadequate preventive measures. However, in this study, the association of GNB with ICU infection is higher than in most of the studies done around the globe in Bosnia (65.2%), India (62%), and Nigeria (50.9%) [12-14]. We found that the prevalence of VAP was highest 70 (43%) followed by CAUTI (39, 24%), CLABSI (25, 15%), SSI (14, 8.6%), non-SSI (7, 4.3%), BSI (5, 3%), and the lowest prevalence was for RTI (3, 1.8%). Similar findings were also reported by Parajul et al. with VAP (53%), but in his study, the second most common is the bloodstream infection (18.8%) which differs from this study [15]. Other reports also describe VAP as the most common healthcare-associated infection in the ICU [16,17].

However, in a previous study, the author pointed to urinary tract infection as the predominant infection, followed by pneumonia and SSI [18]. A study by Datta et al. [19] reported that CLABSI (13.50%) was the most common healthcare-associated infection, followed by UTI (10.75%) and VAP (6.15%). Our one isolate of *S. epidermidis* was reported from CLABSI infection, while the study of Datta et al. did not report any *S. epidermidis* isolates from CLABSI. This variation in clinical types might be due to the differences in the ICU precaution measures and patient population. The most common bacterial isolates reported from our study were *K. pneumoniae* (39, 24%), followed by *A. baumannii* (35, 21.4%) and *P. aeruginosa* (25, 15.3%); other authors reported similar findings [20].

In our study, the prevalence of non-fermenters was high (60, 36.8%, 60/163). Of the non-fermenters, the *A. baumannii* shows the highest occurrence in VAP 18 (51.4%, 18/35) and SSI 9 (25.7%, 9/35) and the least in non-SSI and BSI (2.8%, 1/35) respectively. *P. aeruginosa* shows an equal distribution in VAP, and CAUTI 9 (36%, 9/25) with the least in non-SSI 1 (4%, 1/25), and similar findings were reported in other studies [15,21,22]. A single

isolate of *Burkholderia cepacia* was reported in VAP in our ICU setting. Our study claimed that *P. aeruginosa* produces three types of beta-lactamases, and *A. baumannii* was also the producer of beta-lactamases, including MBL, ESBL, and AmpC, while *K. pneumoniae* isolates did not produce MBL. Interestingly, a study reported just the opposite, while *K. pneumoniae* produced MBL and *P. aeruginosa* isolates did not produce MBL and AmpC [23]. However, in this study, 42 (42.4%, 42/99) of the ICU patients had infections with ESBL-producing gram-negative bacilli, of which the most common ESBL producer is *K. pneumoniae* (22, 56%, 22/42), followed by *A. baumannii* (12, 34.2%, 12/44) and *P. aeruginosa* (8, 32%, 8/44). The prevalence of ESBL in this study was higher than those reported from different parts of the world like India (35.2%), Nepal (28.2%), Qatar (26%), and France (25%) [24-26]. A highly significant associated risk factor related to our study was endotracheal tube (ET) intubation, prolonged mechanical ventilation, and the presence of comorbid condition as a chronic obstructive pulmonary disease (COPD). Duration of ICU stay was also an important risk factor associated with ICU infection, which is in agreement with the study of Confalonieri et al. [27]. But there is another disagreement with the finding that COPD is an associated risk factor for ICU patients in developing healthcare-associated infection [20]. As reported earlier, *A. baumannii* was the most common isolate, followed by P. aeruginosa in VAP patients [28].

Gram-negative bacteria were found to be more common with healthcare-associated infections than gram-positive bacteria. Post-operative state and prior hospitalization have not been shown to significantly influence healthcare-associated infections in ICU.

Public health problems can be triggered by hospital strains spreading into the community [29,30]. The isolation of antibiotic-resistant organisms in intensive care units is particularly high [27,31]. The wide prevalence of nosocomial infections is due to poor hygiene in hospitals, negligence on the part of medical personnel, and non-compliance with antibiotic stewardship guidelines. Hence infections caused by microorganisms continue to be one of the leading causes of hospital deaths worldwide [24,32].

## 5. Conclusions

This study presented a high prevalence of *K. pneumoniae* in ICU patients, followed by *A. baumannii* with VAP as the most common clinical type, followed by CAUTI. ESBL production was the highest in comparison to AmpC and MBL. Hence, proper use of antibiotics, continuous monitoring of drug sensitivity patterns, and taking all precaution measures to prevent beta-lactamases producing organisms in the clinical settings are crucial and significant factors to fend off life-threatening infections and for a better outcome.

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**Informed Consent Statement:** Patients were informed about the research, and informed consent was taken from them.

**Data Availability Statement:** The original and raw data used and reported in this study is available with the first author and corresponding author.

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