ORIGINAL ARTICLE

# Characterization of β-Lactamase Genes among Multidrug- and Extensively Drug-Resistant *Acinetobacter* and Enterobacteriaceae Species Causing Infections in Critically Ill Patients

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***Key words:***
***Antimicrobial, Resistance, Gram-negative, Carbapenemase, Carbapenems***

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## ABSTRACT

***Background:*** *Multidrug resistant infection is the leading cause of ICU morbidity and mortality. Gram-negative bacilli are the most prevalent pathogens with Acinetobacter being the most resistant.* ***Objectives:*** *Phenotypic and genotypic characterization of multidrug- and extensively drug-resistant Acinetobacter and Enterobacteriaceae species causing infections in critically ill patients with detection of targeted β-lactamase genes and its impact on patients' morbidity and mortality.* ***Methodology:*** *Antibiotic susceptibility testing was done. Genes for class A and class B β-lactamases were detected by multiplex polymerase chain reaction (PCR) and 5 carbapenemases genes were detected by real time PCR using GeneXpert Carba-R system.* ***Results:*** *16.6 % of A. baumannii were MDR, 50% were XDR, 50% of K. pneumoniae were XDR and 100% of P. stuartii were XDR. Detected genes included* $bla_{SHV}$, $bla_{CTX-M}$, $bla_{GIM-1}$, $bla_{SIM-1}$, $bla_{OXA-48}$ *and* $bla_{NDM-1}$. ***Conclusion:*** *MDR/XDR infections had a significant impact on patients' mortality. Molecular epidemiology is crucial to guide infection control committees about preventive measures that can contain MDR/XDR infections and prevent their detrimental effect on patients' outcome.*

## INTRODUCTION

One of the biggest risks for critically ill patients in intensive care units (ICUs) is multidrug resistant organisms (MDROs), which restricts treatment options, leads to poor clinical outcomes, and drives up healthcare costs. Gram-negative (GN) bacteria that are extensively drug resistant (XDR) and multi-drug resistant (MDR) are currently endemic[1].

Adult ICU patients with infections and at least one organ dysfunction had high mortality rates because of complications from sepsis[2]. Advanced age, comorbidity (such as diabetes, kidney failure, respiratory failure, etc.) and patient ward (such as ICU, emergency department, etc.) are all risk factors for sepsis[3].

The American Chest Diseases Association/Society of Critical Care Medicine defined sepsis as "life-threatening organ dysfunction that develops as a result of impaired host response to infection". To be diagnosed with sepsis, it is necessary to have both a confirmed infection and a sequential organ failure assessment (SOFA) score of 2 or above[4]. GN bacteria have increasingly become a factor in sepsis due to the widespread use of antibiotics[5]. GN bacilli were found to be the most common bacterial strains in ICUs in Saudi Arabia with *Acinetobacter* being classified as the most resistant strain[6].

Patients admitted to ICU from the emergency room had high rates of carbapenem-resistant Enterobacterales (CRE) colonization[7]. A taxonomy change was made in 2020 to use the name "Enterobacterales" as the name of a new scientific order to replace "*Enterobacteriaceae*," which became, along with other GN bacilli, a family within the "Enterobacterales" order[8].

The use of carbapenem, upper digestive endoscopy, and transfer from another hospital were all risk factors for CRE infections. In the *Enterobacteriaceae*, including *Enterobacter*, *Klebsiella*, *Escherichia coli (E. coli)*, and other opportunistic species such as *Serratia*, *Acinetobacter*, and *Pseudomonas*, novel extended-spectrum -lactamases (ESBLs) and carbapenemases have been reported[9].

Ventilator-associated pneumonia (VAP) and burn site infections, are commonly caused by *Acinetobacter baumannii (A. baumannii)* which can exhibit resistance to carbapenems, aminoglycosides, and cephalosporins

of the third and fourth generations[10]. *A. baumannii* infections caused by MDR or XDR strains in VAP and burn patients causes significant morbidity and mortality[11]. Also, the rise of *Klebsiella pneumoniae (K. pneumoniae)* strains resistant to broad-spectrum antimicrobial drugs poses a significant risk to public health[12].

Hence, the detection of MDR and XDR strains is a pivotal step for infection control committees to control the spread of such organisms. Molecular characterization of MDR and XDR pathogens with different methods such as polymerase chain reaction (PCR) can be of great help for determining the genetic relatedness of these strains and their possible nosocomial infection origin.

The aim of this study is phenotypic and genotypic characterization of multidrug- and extensively drug-resistant *Acinetobacter* and Enterobacteriaceae species causing infections in critically ill patients with detection of targeted β-lactamase genes and its impact on patients' morbidity and mortality.

## METHODOLOGY

A prospective cohort study was conducted at the King Abdul-Aziz Hospital (KAAH) (Jeddah, Saudi Arabia) during August and September, 2021.

**Inclusion criteria:**

Recruited cases included critically ill patients with SOFA score > 2 admitted to ICU and burn unit. Microbiological samples were collected from patients showing clinically suspected infections according to criteria for specific types of infections in the acute care setting, Centers for Disease Control (CDC)[13]. Microbiological cultures were done for isolates identification and antimicrobial susceptibility according to Clinical and Laboratory Standards Institute (CLSI)[14]. MDR and XDR *A. baumannii* and *Enterobacteriaceae* species were screened for targeted β-lactamase genes using multiplex PCR assays. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all except two or less antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)[15].

Clinical data were collected from electronic patients' files including signs and symptoms, co-morbidities and laboratory data such as kidney function, liver function and complete blood counts (CBC). Patients were followed up for determining patients' outcome as survivor (ICU Discharge) or non-survivor (ICU Death).

**Exclusion criteria:**

Patients with infections caused by other causative organisms other than *A. baumannii* and *Enterobactreacea* species were excluded and isolates were not processed further.

**Ethical Considerations:**

The study was approved by the Research and Studies Department – Jeddah Health Affairs Institutional Review Board (IRB) registration number with KACST, KSA: H-02-J-002 research number 1528 and in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and Good Clinical Practice guidelines. The clinical samples were collected as a part of routine microbiological investigations for critically ill patients. Patients or guardians were informed about data collection and patients' data confidentiality was maintained.

**Patients' groups:**

Patients fulfilling inclusion criteria were categorized into 3 groups:

- Group I: Critically ill patients with infections caused by MDR and XDR *A. baumannii* and *Enterobacteriaceae* species.
- Group II: Critically ill patients with infections caused by susceptible *A. baumannii* and *Enterobacteriaceae* species.
- Group III: Critically ill patients without clinically suspected infection.

**Samples collection:**

Various clinical samples of sputum, urine, blood, burn wound and surgical wound swabs were collected from 42 critically ill patients with clinically suspected infections.

**Culture, identification and antimicrobial susceptibility testing:**

Bacterial isolation and identification from sputum, urine, and other samples was performed by inoculating one loopful of each sample onto blood, chocolate and MacConkey agar plates using a sterile disposable plastic loop. Gram-negative pathogens isolated after aerobic incubation at 37˚C for 24 hours were thoroughly identified based on the colony morphology, Gram's staining and oxidase test.

Antimicrobial sensitivity testing was done using Kirby–Bauer disc diffusion method according to CLSI[14] using the following antibiotic discs (Mast Group, UK): ampicillin (Amp-10 µg), amoxicillin/clavulanate (AUG-20/10 µg), cefazolin (CZ-30 µg), cefepime (CPM-30 µg), ceftazidime (CAZ-30 µg), ceftriaxone (CRO-30 µg), cefuroxime (CXM-30 µg), imipenem (IMI-10 µg), meropenem (MEM-10 µg), piperacillin–tazobactam (PTZ-100/10 µg), ciprofloxacin (CIP-5 µg), levofloxacin (LEV-5 µg), gentamicin (GM-10 µg), amikacin (AK-30 µg), aztreonam (ATM-30 µg) and trimethoprim/sulfamethoxazole (TS-1.25/23.75 µg).

Full identification of bacterial isolates and minimal inhibitory concentration (MIC) for different antibiotics were determined using the automated systems BD Phoenix (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) using Gram negative Panel (GN94), and MicroScan WalkAway (Dade Behring INC. West Sacramento, CA, USA) using NBC50 panel including

nitrofurantoin, colistin and tigecycline antibiotics. Quality control strains included *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 as recommended by CLSI[14].

**Multiplex PCR analysis for class A and class B β-lactamase genes:**

For molecular detection of class A and class B β-lactamase genes, MDR *A. baumannii*, XDR *A. baumannii*, XDR *K. pneumoniae*, XDR *Providencia stuartii (P. stuartii)* were tested using multiplex PCR (M-PCR). Isolates were sub-cultured and stored in 40% sterile glycerol-broth medium at −80 °C in preparation for subsequent analysis. DNA extraction was done using QIAamp DNA Micro Kit, (Qiagen, Hilden, Germany). Primers were manufactured by Macrogen Genomics (Seoul, South Korea). The M-PCR assay was performed using the GoTaq® Green Master Mix (M712) (Promega, USA) and Veriti™ 96-Well Fast Thermal Cycler (Thermo Fisher Scientific, USA).

M-PCR for class A β-lactamase genes ($bla_{SHV}$, $bla_{CTX-M}$ and $bla_{TEM}$) was performed according to the method of *Ehlers et al.*[16]. Primers, annealing temperatures, expected amplicon sizes are given in table1. The 25-µL final reaction mixture consisted of 12.5 µL 2 X GoTaq® Green Master Mix (M712), with l µL from each primer (10 pmol/ml), 5.5 µL nuclease-free water and a volume of 4 µL of the prepared DNA template. The cycling conditions were denaturation at 94 °C for 10 min, 30 cycles of denaturation at 94 °C for 1 min, followed by annealing at 60 °C for 1 min, extension at 72 °C for 1 min and a final extension step at 72 °C for 7 min. Gel electrophoresis was done on 2% agarose gel for amplicons visualization against 100-bp ladder (Promega, USA).

**Table 1: Primers for class A β-lactamases Multiplex PCR:**

| **Primer name** | **SHV** | **Length** |
|---|---|---|
| **Forward primer** | F: 5’ ATGCGTTATATTCGCCTGTG -3’ | **20** |
| **Reverse primer** | R: 5’- TGCTTTGTTCGGGCCAA -3’ **-3'** | **17** |
| **Product length** | 747 bp | |
| **Annealing Temp** | 60°C | |
| **Primer name** | **CTX-M** | **Length** |
| **Forward primer** | F: 5’ ATGTGCAGYACCAGTAARGTKATGGC -3’ | **26** |
| **Reverse primer** | R: 5’- TGGGTRAARTARGTSACCAGAAYCAGCG -3’ **-3'** | **28** |
| **Product length** | 593 bp | |
| **Annealing Temp** | 60°C | |
| **Primer name** | **TEM** | **Length** |
| **Forward primer** | F: 5’ TCGCCGCATACACTATTCTCAGAATG -3’ | **26** |
| **Reverse primer** | R: 5’- ACGCTCACCGGCTCCAGATTTAT -3’ **-3'** | **23** |
| **Product length** | 445 bp | |
| **Annealing Temp** | 60°C | |

Multiplex PCR for class B β-lactamases (Metallo-*β*-lactamases) genes ($bla_{IMP}$, $bla_{VIM}$, $bla_{GIM-1}$, $bla_{SPM-1}$, and $bla_{SIM-1}$) was done according to *Alkasaby and El Sayed*[17]. The reaction mix consisted of 4 µL of the extracted DNA applied over 12.5 µL 2 X GoTaq® Green Master Mix (M712) (Promega, USA), with l µL from each primer (10 pmol/ml) (reverse and forward primers) (5 µL) and 3.5 µL nuclease-free water. Primers are shown in table 2. The cycling conditions were initial DNA release and denaturation at 94°C for 5 min, followed by 36 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 50 s, followed by a single, final, elongation step at 72°C for 5 min. Gel electrophoresis was done on 2% agarose gel for amplicons visualization against 100-bp ladder (Promega, USA).

**Table 2: Primers for class B β-lactamases Multiplex PCR:**

| **Primer name** | **IMP** | **Length** |
|---|---|---|
| **Forward primer** | F: 5'- GGAATAGAGTGGCTTAAYTCTC -3' | **22** |
| **Reverse primer** | R: 5'- CCAAACYACTASGTTATCT -3' **-3'** | **19** |
| **Product length** | 188 bp | |
| **Annealing Temp** | 52°C | |
| **Primer name** | **VIM** | **Length** |
| **Forward primer** | F: 5'- GATGGTGTTTGGTCGCATA -3' | **19** |
| **Reverse primer** | R: 5'- CGAATGCGCAGCACCAG -3' **-3'** | **17** |
| **Product length** | 390 bp | |
| **Annealing Temp** | 52°C | |
| **Primer name** | **GIM-1** | **Length** |
| **Forward primer** | F: 5'- TCGACACACCTTGGTCTGAA -3' | **20** |
| **Reverse primer** | R: 5'- AACTTCCAACTTTGCCATGC -3' **-3'** | **20** |
| **Product length** | 477 bp | |
| **Annealing Temp** | 52°C | |
| **Primer name** | **SPM-1** | **Length** |
| **Forward primer** | F: 5'- AAAATCTGGGTACGCAAACG -3' | **20** |
| **Reverse primer** | R: 5'- ACATTATCCGCTGGAACAGG -3' **-3'** | **20** |
| **Product length** | 271 bp | |
| **Annealing Temp** | 52°C | |
| **Primer name** | **SIM-1** | **Length** |
| **Forward primer** | F: 5'- TACAAGGGATTCGGCATCG -3' | **19** |
| **Reverse primer** | R: 5'- TAATGGCCTGTTCCCATGTG -3' **-3'** | **20** |
| **Product length** | 570 bp | |
| **Annealing Temp** | 52°C | |

Real-time PCR for five carbapenemase genes was performed using the Xpert Carba-R Assay, performed on the GeneXpert Instrument Systems, (Cepheid, Sunnyvale, CA, USA) for the detection of $bla_{KPC}$ (class A β-lactamases), $bla_{NDM-1}$, $bla_{VIM}$, $bla_{IMP}$ (class B β-lactamases), and $bla_{OXA-48}$ (class D β-lactamases). A bacterial suspension (0.5 McFarland) was prepared. The 0.5 McFarland suspension was vortexed. Using a 10 µL loop, 10 µL of the 0.5 McFarland suspension was transferred to a 5 mL sample reagent vial which was capped and vortexed at high speed for 10 seconds. Using the transfer pipette 1.7 ml of the vortexed Xpert Sample Reagent was transferred to the cartridge and placed in the GeneXpert instrument within 30 minutes of adding the sample to the cartridge.

For molecular studies, K. pneumoniae ATCC 2146, K. pneumoniae ATCC 1705, K. pneumoniae NCTC 13442 were used as positive controls and K. pneumoniae ATCC 25955 was used as negative control.

**Statistical Analysis:** The collected data was revised and tabulated using Statistical package for Social Science (SPSS 25). Mean, Standard deviation (± SD) for numerical data. Frequency and percentage of categorical data. ANOVA test was used to assess the statistical significance of the difference between more than two study group means. Post Hoc Test is used for comparisons of all possible pairs of group means. Chi-Square test and Fisher's exact test were used to examine the relationship between two qualitative variables.

## RESULTS

The study was conducted on 73 critically ill patients (32 males and 41 females) with SOFA score > 2 admitted to ICU and burn unit KAAH (Jeddah, Saudi Arabia) during August and September, 2021. Twelve patients were excluded from the study with sites of infection that showed the growth of *Staphylococcus aureus (S. aureus)* (n=5), coagulase negative Staphylococci (CONS) (n=4) and Methicillin-resistant *S. aureus (MRSA)* (n=3). Patients fulfilling inclusion criteria (n=61) were categorized in 3 groups: Group I included critically ill patients with infections caused by MDR and XDR *A. baumannii* and *Enterobacteriaceae* species (n=22). Group II included critically ill patients with infections caused by susceptible *A. baumannii* and

*Enterobacteriaceae* species (n=20). Group III included critically ill patients without clinically suspected infection (n=19). No statistically significant difference was observed between patients' groups as regards age and sex (table 3). Co-morbidities included diabetes mellitus (47 patients, 77.1%), hypertension (56 patients, 91.8%) and renal insufficiency (9 patients, 14.7%).

**Table 3: Comparison between 3 groups regarding age and sex:**

| | | Group I | | Group II | | Group III | | Test of significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | value | p value | sig. |
| Gender | Male | 10 | 45.4% | 9 | 45% | 8 | 42.2% | $X^2 = 0.04$ | 0.982 | NS |
| | Female | 12 | 54.6% | 11 | 55% | 11 | 57.8% | | | |
| | Total | 22 | 100% | 20 | 100% | 19 | 100% | | | |
| | | Mean | SD | Mean | SD | Mean | SD | ANOVA | | |
| Age | | 62.38 | 12.74 | 64.07 | 14.01 | 60.44 | 11.54 | F = 0.31 | 0.738 | NS |

*NS non-significant, Sig. Significance, N number*

Statistical analysis showed a significant difference between the three studied groups as regards white blood cells (WBCs) count and neutrophil (NU) count with higher counts observed in patients of group I and group II compared to patients of group III. No statistically significant difference was observed between the three studied groups as regards platelets count, hemoglobin concentration (HB), aspartate transaminase (AST) serum level, alanine aminotransferase (ALT) serum level, bilirubin, serum creatinine (S Cr.) and blood urea nitrogen (BUN) (table 4).

**Table 4: Comparison between 3 groups regarding laboratory findings:**

| | Group I | | Group II | | Group III | | ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | F | p value | sig. |
| **WBCs** | 15.83 | 4.53 | 15.79 | 4.52 | 7.74 | 1.64 | 24.05 | <0.001* | S |
| **Nu** | 13.23 | 4.07 | 14.56 | 4.55 | 6.23 | 1.58 | 23.99 | <0.001* | S |
| **Platelets** | 246.33 | 79.66 | 309.71 | 85.03 | 289.94 | 114.94 | 2.34 | 0.107 | NS |
| **HB** | 12.41 | 1.67 | 13.17 | 1.82 | 13.11 | 2.16 | 1.02 | 0.368 | NS |
| **AST U/L** | 44.92 | 8.95 | 41.71 | 8.30 | 42.94 | 6.68 | 0.73 | 0.486 | NS |
| **ALT U/L** | 44.13 | 9.71 | 38.29 | 7.23 | 44.25 | 7.90 | 2.43 | 0.099 | NS |
| **Bilirubin** | 1.17 | 0.45 | 0.99 | 0.18 | 1.11 | 0.34 | 1.04 | 0.362 | NS |
| **S. Cr** | 116.21 | 60.28 | 92.07 | 37.11 | 83.44 | 22.98 | 2.70 | 0.077 | NS |
| **BUN** | 6.75 | 2.81 | 5.16 | 1.66 | 5.63 | 2.22 | 2.21 | 0.120 | NS |

**Post hoc test: group 1 vs 2 (NS), group 1 vs 3 (S) and, group 2 vs 3 (S)*

Samples, from patients with clinically suspected infection (n=42) from different infection sites, included sputum (28.6%, n=12), urine (23.8%, n=10), blood (21.4%, n=9), burn wound swab (16.7%, n=7), and surgical wound swab (9.5%, n=4). They showed the growth of *A. baumannii* (18 isolates, 42.9%), *K. pneumoniae* (16 isolates, 38.1%), *E. coli* (6 isolates, 14.3%) and *P. stuartii* (2 isolates, 4.7%). Antimicrobials (Abs) susceptibility testing results are shown in table 5.

Among the detected isolates of *A. baumannii* (n=18), 16.6 % were MDR (n=3) and 50 % were XDR (n=9). As regards *K. pneumoniae* isolates (n=16) 50% were XDR (n=8), while the detected isolates of *P. stuartii* (n=2) were both XDR. *E. coli* isolates were 100% sensitive to aminoglycosides, carbapenems, colistin and tigecycline. None of the *E. coli* isolates was MDR or XDR.

**Table 5: Antimicrobial susceptibility testing results:**

| Species / Antibiotics | Sensitivity | *A. baumannii* Total = 18 isolates | *K. pneumoniae* Total = 16 isolates | *E. coli* Total = 6 isolates | *P. stuartii* Total = 2 isolates |
|---|---|---|---|---|---|
| | | No. (%) | No. (%) | No. (%) | No. (%) |
| **Ceftazidime** | **S** | 6 (33.3%) | 4 (25%) | 3 (50%) | 2 (100%) |
| | **I** | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 12 (66.7%) | 12 (75%) | 3 (50%) | 0 (0%) |
| **Ceftriaxone** | **S** | 6 (33.3%) | 8 (50%) | 3 (50%) | 0 (0%) |
| | **I** | 0 (0%) | 0 (0%) | 1 (16.7%) | 0 (0%) |
| | **R** | 12 (66.7%) | 8 (50%) | 2 (33.3%) | 2 (100%) |
| **Cefepime** | **S** | 7 (38.8%) | 6 (37.5%) | 4 (66.7%) | 0 (0%) |
| | **I** | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 11 (61.2%) | 10 (62.5%) | 2 (33.3%) | 2 (100%) |
| **Cefazolin** | **S** | - | 7 (43.7%) | 3 (50%) | 0 (0%) |
| | **I** | - | 1 (6.3%) | 0 (0%) | 0 (0%) |
| | **R** | - | 8 (50%) | 3 (50%) | 2 (100%) |
| **Cefuroxime** | **S** | - | 8 (50%) | 3 (50%) | 0 (0%) |
| | **I** | - | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | - | 8 (50%) | 3 (50%) | 2 (100%) |
| **Ampicillin** | **S** | - | 3 (18.7%) | 3 (50%) | 0 (0%) |
| | **I** | - | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | - | 13 (81.3%) | 3 (50%) | 2 (100%) |
| **Amoxicillin/Clavulanate** | **S** | - | 8 (50%) | 3 (50%) | 0 (0%) |
| | **I** | - | 2 (12.5%) | 0 (0%) | 0 (0%) |
| | **R** | - | 6 (37.5%) | 3 (50%) | 2 (100%) |
| **Piperacillin–Tazobactam** | **S** | 4 (22.2%) | 9 (56.3%) | 5 (83.3%) | 0 (0%) |
| | **I** | 2 (11.1%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 12 (66.7%) | 7 (43.7%) | 1 (16.7%) | 2 (100%) |
| **Imipenem** | **S** | 4 (22.2%) | 9 (56.25%) | 6 (100%) | 0 (0%) |
| | **I** | 1 (5.5%) | 2 (12.5%) | 0 (0%) | 0 (0%) |
| | **R** | 13 (72.3%) | 5 (31.25%) | 0 (0%) | 2 (100%) |
| **Meropenem** | **S** | 5 (27.7%) | 11 (68.75%) | 6 (100%) | 0 (0%) |
| | **I** | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 13 (72.3%) | 5 (31.25%) | 0 (0%) | 2 (100%) |
| **Aztreonam** | **S** | - | 8 (50%) | 6 (100%) | 2 (100%) |
| | **I** | - | 1 (6.3%) | 0 (0%) | 0 (0%) |
| | **R** | - | 7 (43.7%) | 0 (0%) | 0 (0%) |
| **Gentamicin** | **S** | 4 (22.2%) | 4 (25%) | 6 (100%) | 0 (0%) |
| | **I** | 3 (16.7%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 11 (61.2%) | 12 (75%) | 0 (0%) | 2 (100%) |
| **Amikacin** | **S** | 6 (33.3%) | 5 (31.3%) | 6 (100%) | 0 (0%) |
| | **I** | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 12 (66.7%) | 11 (68.7%) | 0 (0%) | 2 (100%) |
| **Ciprofloxacin** | **S** | 3 (16.6%) | 5 (31.3%) | 5 (83.3%) | 0 (0%) |
| | **I** | 2 (11.1%) | 3 (18.7) | 0 (0%) | 0 (0%) |
| | **R** | 13 (72.3%) | 8 (50%) | 1 (16.7%) | 2 (100%) |
| **Levofloxacin** | **S** | 2 (11.1%) | 5 (31.25%) | 4 (66.7%) | 0 (0%) |
| | **I** | 1 (5.5%) | 2 (12.5%) | 1 (16.7%) | 0 (0%) |
| | **R** | 15 (83.3%) | 9 (56.25%) | 1 (16.7%) | 2 (100%) |
| **Trimethoprim/ Sulfamethoxazole** | **S** | 8 (44.4%) | 4 (25%) | 6 (100%) | 0 (0%) |
| | **I** | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 10 (55.6%) | 12 (75%) | 0 (0%) | 2 (100%) |
| **Nitrofurantoin** | **S** | - | Total = 4 | 0(0%) 6 (100%) | - |
| | **I** | - | | 1(25%) 0 (0%) | - |
| | **R** | - | | 3(75%) 0 (0%) | - |
| **Colistin** | **S** | 17 (94.5%) | 13 (81.3%) | 6 (100%) | 0 (0%) |
| | **I** | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| | **R** | 1 (5.5%) | 3 (18.7) | 0 (0%) | 2 (100%) |
| **Tigecycline** | **S** | - | 11 (68.75%) | 6 (100%) | - |
| | **I** | - | 3 (18.75%) | 0 (0%) | - |
| | **R** | - | 2 (12.5%) | 0 (0%) | - |

As regards molecular detection of class A and class B β-lactamase genes among MDR and XDR isolates, *A. baumannii* (n=12), *K. pneumoniae* (n=8), *P. stuartii* (n=2), multiplex PCR detected 2 class A β-lactamase genes; $bla_{SHV,}$ gene was detected in all of the 8 XDR *K. pneumoniae* isolates (100%) (figure1) and $bla_{CTX-M}$ gene was detected in 10 isolates out of 12 MDR / XDR *A. baumannii* isolates (83.3%) (figure 2). Also, 2 class B β-lactamase genes were detected; $bla_{GIM-1}$ gene was detected in 2 out of 12 MDR / XDR *A. baumannii* isolates (16.6%) and $bla_{SIM-1}$ gene was detected in one XDR *A. baumannii* isolate (8.3%) which showed simultaneous detection of the 2 genes (figure 3). None of the targeted genes was detected in *P. stuartii* isolates

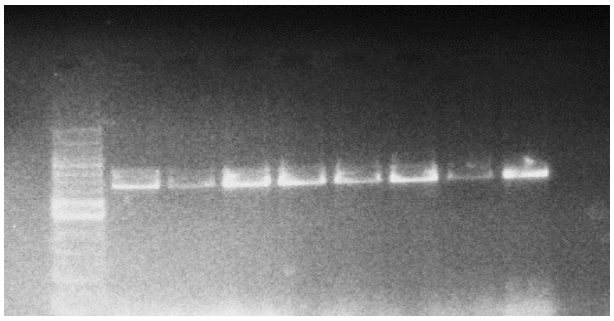

**Fig. 1:** Gel electrophoresis for class A β-lactamase genes M-PCR product showing detection of *bla*$_{SHV}$ (747bp) in 8 XDR *K. pneumoniae* isolates.

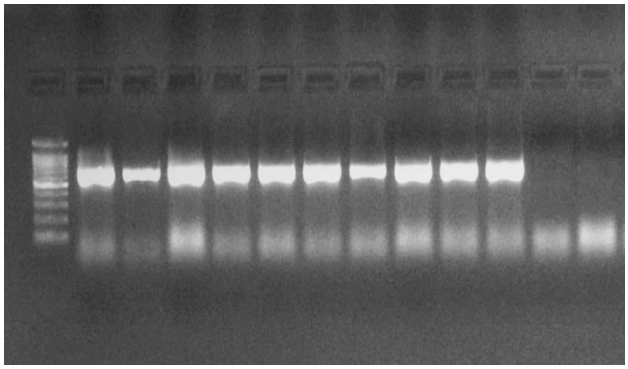

**Fig. 2:** Gel electrophoresis for class A β-lactamase genes M-PCR products showing detection of *bla*$_{CTX-M}$ (593bp) in 10 MDR / XDR *A. baumannii* isolates.

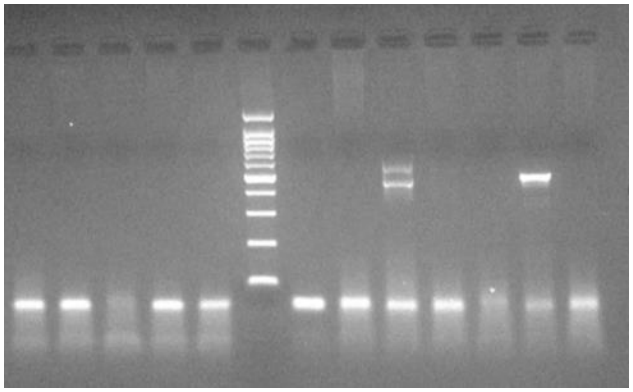

**Fig. 3 :** Gel electrophoresis for class B β-lactamase genes M-PCR products showing detection of *bla*$_{GIM-1}$ (477bp) in 2 MDR / XDR *A. baumannii* isolates and *bla*$_{SIM-1}$ (570bp) in 1 XDR *A. baumannii* isolate.

GeneXpert system analysis for carbapenemase genes detected both of *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ in 2 out of 8 XDR *K. pneumoniae* isolates (25%) (figure 4), *bla*$_{OXA-48}$ alone was detected in 2 out of 8 XDR *K. pneumoniae* isolates (25%) (figure 5) and *bla*$_{NDM-1}$ alone was detected in 1 out of 8 XDR *K. pneumoniae* isolates (12.5%) (figure 6). None of the targeted carbapenemases genes was detected among *A. baumannii* or *P. stuartii* isolates.

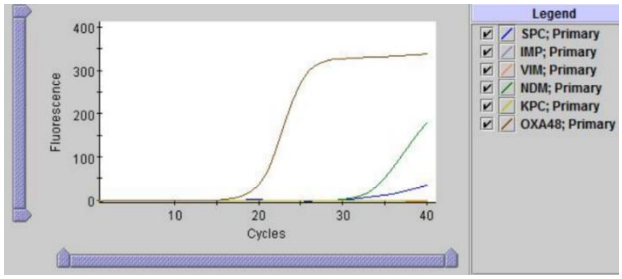


**Fig. 4:** Amplification curve showing simultaneous detection of *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ genes.

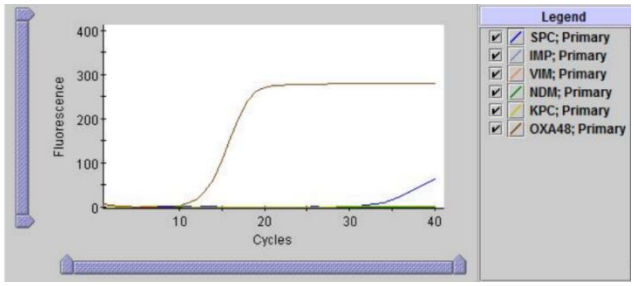


**Fig. 5:** Amplification curve showing detection of *bla*$_{OXA-48}$ gene.

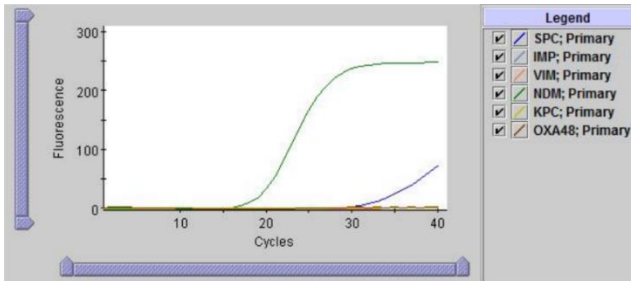


**Fig. 6:** Amplification curve showing detection of *bla*$_{NDM-1}$ gene.

As regards comparing outcome between the three patients' groups, table 6 shows a statistically significant difference between patients of group I (Critically ill patients with MDR and XDR infections) with 40.9% non- survivors and patients of group III (Critically ill patients without clinically suspected infections) with 5.2% non- survivors. No statistically significant difference is shown between other patients' groups as regards number of non- survivors.

**Table 6: Comparison between 3 groups regarding outcome:**

| | | Group I | | Group II | | Group III | | Fisher exact test | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | value | p value | sig. |
| Outcome | Non survivor | 9 | 40.90%[a] | 4 | 20.00%[a,b] | 1 | 5.20%[b] | 7.27 | 0.028 | S |
| | Survivor | 13 | 59.10%[a] | 16 | 80.00%[a,b] | 18 | 94.80%[b] | | | |
| | Total | 22 | 100% | 20 | 100% | 19 | 100% | | | |

**Post hoc test: group 1 vs 2 (NS), group 1 vs 3 (S) and, group 2 vs 3 (NS)*

## DISCUSSION

Since ICUs house immunocompromised critically sick patients, they pose a special danger for multidrug-resistant infections. ICUs also provide a favorable setting for MDROs multiplication and survival amongst substantial antibiotic pressure. This was highlighted by *Banerjee et al.*[18]who found that over 70% of ICU patients were receiving antibiotics for therapeutic or preventative purposes.

The present study was conducted on 73 critically ill patients (32 males and 41 females) with SOFA score > 2 admitted to ICU and burn unit KAAH (Jeddah, Saudi Arabia). Twelve patients were excluded from the study. Patients fulfilling inclusion criteria (n=61) were categorized into 3 groups with no statistically significant difference as regards age and sex observed between patients' groups.

Samples were collected from different infection sites including sputum (28.6%), urine (23.8%), blood (21.4%), burn wound swab (16.7%), and surgical wound swab (9.5%). This agreed with the review of *Alharbi et al.*[6] which reported that the most common site of infection in patients admitted to general ICUs was respiratory tract, followed by urinary tract infections and bloodstream infections. The predominant species, isolated during the present study, were *A. baumannii* (18 isolates, 42.9%) followed by *K. pneumoniae* (16 isolates, 38.1%), *E. coli* (6 isolates, 14.3%) and *P. stuartii* (2 isolates, 4.7%). Similar results were demonstrated by *Han et al.*[19] as they reported that *A. baumannii* was the most prevalent species representing 35.97% of total isolates.

*A. baumannii* showed lowest antimicrobial resistance to colistin (Polymyxin E) (5.5%) and highest resistance to levofloxacin (83.3%). *A. baumannii* showed resistance of 61.2% - 72.3% for all tested cephalosporins, carbapenems and aminoglycosides and 55.6% for trimethoprim/sulfamethoxazole. This was in accordance with *Shi et al.*[15] who reported that *A. baumannii* was resistant to carbapenems, aminoglycosides, most cephalosporins and sulfa containing antibiotics with resistance rates of > 75%. They also reported *A. baumannii* polymyxin sensitivity of 96.67%.

For *K. pneumoniae*, in the present study, highest sensitivity was observed with colistin (81.3%) followed by tigecycline (68.7%) and the lowest sensitivity was observed with ampicillin (18.7%) followed by gentamicin and trimethoprim/sulfamethoxazole (25% each). *K. pneumoniae* showed resistance of ≥ 50% towards all tested cephalosporines, 43.7% towards piperacillin–tazobactam and 37.5% towards amoxicillin/clavulanate. Similarly, *Han et al.*[19] reported that 9.42% of the detected isolates were *K. pneumoniae* and it showed resistance to most of the tested antibiotics.

*P. stuartii* isolates, in this study, showed 100% sensitivity to ceftazidime and aztreonam and 100% resistance to all other antibiotics. However, *Liu et al.*[20] observed that *P. stuartii* showed sensitivity to imipenem and amikacin and resistance to most of the beta- lactam antibiotics. They also reported that *P. stuartii* was sensitive to cefepime, levofloxacin and ciprofloxacin.

In the present study, *E. coli* isolates were 100% sensitive to aminoglycosides, carbapenems, colistin and tigecycline. *E. coli* showed 50% resistance to most of the tested cephalosporins and other penicillin antibiotics. None of the *E. coli* isolates was MDR or XDR. This was in accordance with the results of *Han et al.*[19] as they reported that 21.79% of their tested isolates were *E. coli* which showed sensitivity to most of the tested antibiotics. However, *Miao and colleagues*[21] reported that all the isolates they tested, including *E. coli*, were resistant to most of the tested β-lactam antibiotics.

The present study showed that, among the detected isolates of *A. baumannii*, 16.6 % were MDR and 50% were XDR. In the study by *Banerjee et al.*[18] they reported higher resistance rates with 88.02% for MDR *A. baumannii* and 61.97% for XDR *A. baumannii*. They attributed the predominance of MDR *A. baumannii*, as a cause of ICU infections, to its ability of prolonged survival in ICU environment and its ability to acquire antibiotic resistance under the pressure of extensive antibiotic use in ICU.

Lower antibiotic resistance rate observed in the present study can be attributed to the concurrent MDROs control project started 4 years earlier. Infection prevention measures were implemented in ICU to reduce MDROs incidence through a CDC based two-

tiered MDROs control and antimicrobial stewardship programs[22].

Results of the present study showed that 50% of the *K. pneumoniae* isolates were XDR and 100% of the *P. stuartii* were XDR. The study of *Bitew and* Tsige[23] reported that, among *K. pneumoniae* isolates, 51.8% were MDR. Also, *Godebo et al.*[24] reported that 75% of *Providencia* isolates were multi-drug resistant. Similarly, the results of *Alkofide et al*[25] showed that 60% of the tested Enterobacteriaceae were either MDR or XDR strains.

As regards class A β-lactamase genes among MDR / XDR isolates *A. baumannii* (n=12), XDR *K. pneumoniae* (n=8) and XDR *P. stuartii* (n=2), multiplex PCR detected 2 class A β-lactamase genes; $bla_{SHV,}$ gene was detected in all the 8 XDR *K. pneumoniae* isolates (100%). In the same context, in the study of *Monstein et al.*[26], multiplex PCR detected the $bla_{SHV}$ gene in all of the 7 *K. pneumoniae* isolates (100%) included in the study, however it was alone in three *K. pneumoniae* isolates, associated with $bla_{TEM}$ gene in one *K. pneumoniae* isolate, and associated with both $bla_{TEM}$ and $bla_{CTX-M}$ genes in three *K. pneumoniae* isolates.

Also, for *A. baumannii*, results of the present study showed that $bla_{CTX-M}$ gene was detected in 10 isolates out of 12 MDR / XDR *A. baumannii* isolates (83.3%). Lower percentage of $bla_{CTX-M}$ gene positive *A. baumannii* isolates (1.8%) was detected by *Alkasaby and El Sayed*[17]. They also reported that the genes detected with highest frequency among the three studied class A β-lactamase genes were $bla_{TEM}$ gene (2.9%) and $bla_{SHV,}$ gene (2.1%) and that some of the isolates harbored more than one gene.

For class B β-lactamase genes, 2 genes were detected; $bla_{GIM-1}$ gene was detected in 2 out of 12 MDR / XDR *A. baumannii* isolates (16.6%) and $bla_{SIM-1}$ gene was detected in 1 XDR *A. baumannii* isolate out of 12 MDR / XDR *A. baumannii* isolates (8.3%) simultaneously with $bla_{GIM-1}$ gene. Higher percentages were detected for MBLs genes by *Alkasaby and El Sayed*[17], the most frequent genes were $bla_{IMP}$ (95.7%), followed by $bla_{SIM}$ and $bla_{GIM}$ (47.1% and 42.9%, respectively).

Regarding carbapenemase genes, both of $bla_{NDM-1}$ and $bla_{OXA-48}$ were detected in 25% of the XDR *K. pneumoniae* isolates, $bla_{OXA-48}$ alone was detected in 25% of the XDR *K. pneumoniae* isolates and $bla_{NDM-1}$ alone was detected in 12.5% of the XDR *K. pneumoniae* isolates. Similarly, the study of *Poirel et al.*[27] showed that *K. pneumoniae* isolates were producing the carbapenemases OXA-48, NDM-1, and KPC-2. They reported 33.3% *K. pneumoniae* isolates were positive for $bla_{NDM-1}$ and 50% were positive for $bla_{OXA-48}$. None of thier isolates coharbored two carbapenemase genes.

Comparing patients' outcome showed that patients with MDR and XDR infections (group I) had the highest mortality rate (41.7%) with a statistically significant difference when compared to patients without clinically suspected infections (group III) that showed the lowest mortality rate (6.3%). Higher in hospital death rate was observed by *Alkofide et al.*[25] among ICU patients. They reported that 57.3% of the studied *Enterobacteriaceae* isolates were MDR and 3.5% were XDR isolates, with in hospital reported death rate of 84.1% for all the patients.

## CONCLUSION

Gram-negative MDR and XDR bacteria were the predominant pathogens causing infections among critically ill patients in ICU and burn unit with the highest mortality rate among the studied groups. Resistance genes characterization and molecular epidemiology is important for clarifying relatedness between different MDR and XDR isolates and possible common source. Further molecular epidemiology studies involving environmental surfaces screening can help identifying the potential common source of infection for patients in critical care areas. This also can guide infectious disease and infection prevention committees about preventive measures and antimicrobial stewardship programs that should be implemented to limit the spread of MDR and XDR infections and prevent their detrimental effects on critically ill patients.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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