

Antimicrobial Resistance of Gram-Negative Bacilli Causing Infections in Intensive Care Units in Makkah Hospitals- Saudi Arabia

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Abstract: The aim of the present study was to determine the incidence and antimicrobial susceptibility patterns of the most common gram-negative bacteria (GNB) causing infections in the intensive care units (ICUs) of Makkah hospitals. In addition to evaluate the production of extended spectrum- β -lactamases (ESBL) in *Klebsiella pneumoniae* and *Escherichia coli* as well as metallo- β -lactamases (MBL) in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. A total of 509 gram-negative pathogens were isolated from clinical specimens of patients admitted ICUs of Makkah hospitals between September 2009 and March 2010. The specimens were microbiologically investigated by the routine methods, and antibiotic susceptibility was performed by using automated instruments. ESBLs and MBLs were determined according to Clinical and Laboratory Standards Institute (CLSI). The types of ESBLs and MBLs were detected by polymerase chain reaction. *A. baumannii* was the common bacteria (37%) isolated from ICUs, followed by *P. aeruginosa* (29.1%), *K. pneumoniae* (22.8%) and *E. coli* (10.6%). *P. aeruginosa* and *A. baumannii* isolates were highly resistant towards the most antibiotic agents. ESBLs production was identified in 37.1% and 31.5% of *K. pneumoniae* and *E. coli* isolates, respectively, and MBLs in 20.9% of *P. aeruginosa* and 68.6% of *A. baumannii* isolates. In conclusion, GNB cause several nosocomial infections in ICUs patients of Makkah hospitals with high resistant rate to antimicrobial agents.

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1. Introduction

Many studies have indicated that *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* are the most frequently isolated gram-negative bacilli (GNB) from intensive care units (ICU) [1,2]. Emerging antimicrobial-resistant strains of these pathogens included multidrug-resistant *A. baumannii* and *P. aeruginosa*, cephalosporin - or fluoroquinolone-resistant *E. coli*, and extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *E. coli* [3-5]. Among β -lactam antibiotics, carbapenems have been successfully used to evade bacterial resistance, but carbapenem resistance due to the production of metallo- β -lactamases (MBLs) has been increasingly reported, particularly for *P. aeruginosa* and *Acinetobacter spp* [6,7]. In recent years, carbapenem resistance in *P. aeruginosa* and *Acinetobacter spp*. has gradually increased in different parts of the world, and a significant proportion of these carbapenem-resistant isolates have been shown to produce VIM-2- or IMP-1-type MBL [8].

Extended-spectrum β -lactamases (ESBLs) clavulanate-susceptible enzymes that present a wide resistance to penicillins, aztreonam and cephalosporins (except cephamycins) have been previously detected in *K. pneumoniae* and *E. Coli*

[9,10]. ESBL production is often plasmid-mediated, with most being mutants of the classic TEM and SHV enzymes, with one or more amino acid substitutions around the active site. These changes allow the hydrolysis of extended-spectrum cephalosporins (e.g. ceftazidime, cefotaxime) and monobactams (e.g. aztreonam), which remain stable only against classic TEM and SHV enzymes [9,11]. However, it has been reported that the emergence of β -lactamases belonging to other families, such as PER, VEB, CTX-M and/or OXA derivatives, is increasing worldwide [9,12].

Previously, many studies have recorded the prevalence and mechanism of resistance among GNB isolated from ICU patients, particularly for *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *E. coli*. However, few studies have examined the prevalence of GNB in Saudi Arabia. No recent information is thus available regarding the prevalence and types of ESBL and MBL production among GNB in the Makkah region. Therefore, the current study aimed to determine the incidence and antibiotic resistance patterns among GNB (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*) causing infections in patients admitted to ICUs of Makkah hospitals. In addition, the production of ESBLs and its types (TEM, SHV, CTX-m) in *E. coli* and *K. pneumoniae* was investigated. The production of MBLs and its types

(VIM, IMP) in *A. baumannii* and *P. aeruginosa* was also examined.

2. Materials and Methods

Study Design

This prospective study was carried out in the three main tertiary care hospitals in Makkah city: Al-Noor Specialist Hospital (560 beds), Hera General Hospital (276 beds) and King Abdulaziz Hospital (400 beds) between September 2009 to March 2010.

Patients and Clinical Isolates

A total of 509 non-duplicated clinical isolates of GNB (*P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *E. coli*) were collected from 313 patients hospitalised in ICUs during the study period. Data were recorded on individual sheet forms, including age, gender, nationality, etc. The clinical isolates were identified by routine microbiological methods. Antimicrobial susceptibility tests were performed for the all clinical isolates using automated instruments (Phoenix 100 BD, USA, and MicroScan Walkaway 96, Siemens, Germany). Minimum inhibitory concentration (MIC) using commercial E-test MIC strips for imipenem were used for *P. aeruginosa* and *A. baumannii*.

Determination of ESBL and MBL-producing isolates

In order to identify the suspected ESBL-producing isolates among *E. coli* and *K. pneumoniae*, the antimicrobial susceptibility disc diffusion method (according to CLSI) was used for cefotaxime, ceftazidime, cefotrixone (third-generation cephalosporins), ceftaxime (second-generation) and aztreonam [13]. *E. coli* ATCC25922 (susceptible strain) and *K. pneumoniae* ATCC700603 (ESBL-producing strain) were included as quality controls [13]. Double-disk synergy test (DDST) was used as a confirmatory method for suspected ESBL-producing isolates [14]. All *P. aeruginosa* and *A. baumannii* clinical isolates were examined for MBL production as previously described [15]. The detection of ESBL types (*bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*) in *E. coli* and *K. pneumoniae* and MBL types (VIM, IMP) in *P. aeruginosa* and *A. baumannii* was performed for suspected clinical isolates using the PCR amplification technique as described previously [16,17].

Statistical Analysis

All clinical and microbiological outcomes were analysed and assessed using the Statistical Package for Social Sciences IBM SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA).

3. Results

A total of 509 GNB (*P. aeruginosa*, 148; *A. baumannii*, 191; *K. pneumoniae*, 116; *E. coli*, 54) were isolated from clinical specimens obtained from the ICUs of Makkah hospitals. Gram-negative infections in the ICU were distributed among 30 different nationalities. The majority were Saudi individuals

(50.9%), followed by Pakistani (8.8%), Indian (5.9%), Egyptian (5.7%) and Yemeni (5.1%) individuals. The most age group affected by gram-negative infections was above 60 years old (Fig. 1). The majority of gram-negative strains were isolated from sputum (45.6%), followed by urine (13.6%), wound swabs (11.6%), tracheal aspirates (11.4%) and blood (10.4%). The majority of *P. aeruginosa* (47.3%) and *A. baumannii* (41.4%) were isolated from the Al-Noor Specialist Hospital. However, *K. pneumoniae* were mainly isolated from Hera General Hospital (42.2%). Most male patients were infected by *A. baumannii* (63.9%), followed by *P. aeruginosa* (56.1%). However, female patients were more infected by *K. pneumoniae* (55.2%) than males. *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* were the most pathogenic bacteria causing RTIs representing 77.5%, 67.6% and 49.1%, respectively; however *E. coli* most frequently caused UTIs (42.6%), (Table 1).

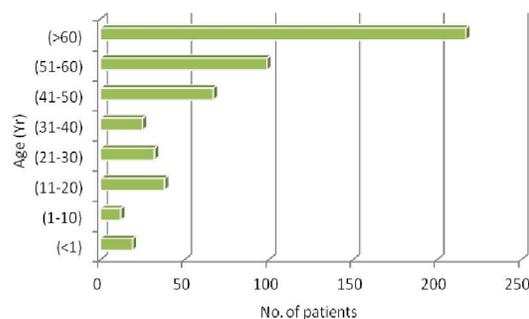


Figure 1. Distribution of common gram-negative bacteria isolated from Makkah hospitals according to patients age

P. aeruginosa and *A. baumannii* showed high resistance towards most antibiotics (Table 2). Some anti-pseudomonal agents showed moderate activity against *P. aeruginosa*, including piperacillin (43.8%), imipenem (43.9%), amikacin (46.7%) meropenem (53.2%) and gentamicin (55.1%). *A. baumannii* isolates were highly resistant to piperacillin (99.2%), ceftazidime (97.2%), ciprofloxacin (96%) and piperacillin/tazobactam (94.5%). The susceptibility rate for *E. coli* and *K. pneumoniae* to imipenem was very high. Gentamycin showed a moderate activity against both *E. coli* and *K. pneumoniae* at 47.1% and 50.9%, respectively (Table 3).

A total of 60 isolates (*K. pneumoniae* 43/37.1%, and *E. Coli*, 17/31.5%) were confirmed as ESBL-producing strains by DDST. The most frequent β -lactamase type present in *K. pneumoniae* was CTX-M (81.4%); however, *E. coli* showed an equal percentage of isolates harbouring TEM and CTX-M (58.8%), (Table 4).

The MIC₅₀ and MIC₉₀ of imipenem against *P. aeruginosa* was 0.5 and 2, respectively. For *A. baumannii*, the MIC₅₀ and MIC₉₀ were above 32, indicating a high resistance rate to imipenem. Isolates that resistant to imipenem and/or meropenem were examined for MBL production. The results revealed that, 31 isolates (20.9%) of *P. aeruginosa* and 131 isolates (68.6%) of *A. baumannii* were MBL-producing isolates. All MBL-positive isolates for both organisms were multidrug-resistant, being resistant to

imipenem, meropenem, ceftazidime, piperacillin/tazobactam, ciprofloxacin and gentamycin.

The PCR results confirmed the presence of *bla*VIM and *bla*IMP in 6 and 7 out of 31 *P. aeruginosa* isolates, respectively. MBL genes were also found in *A. baumannii*, wherein the VIM and IMP types were represented in 22 and 26 isolates, respectively (Table 5).

Table 1. Distribution of *P. aeruginosa*, *A. baumannii*, *K. pneumoniae* and *E. coli* isolated from ICUs according to type of infection.

Isolated Organism	Type of infection										Total
	Septicemia		RTI		UTI		Wound Infection		Genital Infection		
	N	%	N	%	N	%	N	%	N	%	
<i>P. aeruginosa</i>	16	10.8	100	67.6	13	8.8	18	12.2	1	0.7	148
<i>A. baumannii</i>	13	6.8	148	77.5	14	7.3	16	8.4	0	0	191
<i>K. pneumoniae</i>	18	15.5	57	49.1	20	17.2	19	16.4	2	1.7	116
<i>E.coli</i>	10	18.5	10	18.5	23	42.6	9	16.7	2	3.7	54

Table 2. Antibiotic resistance of *P. aeruginosa* and *A. baumannii* to antimicrobial agents

Antimicrobial agents	<i>P. aeruginosa</i>			<i>A. baumannii</i>		
	Total	N	%	Total	N	%
Amikacin	135	63	46.7	168	156	92.9
Cefepime	132	91	68.9	154	143	92.9
Ceftazidime	116	73	62.9	176	171	97.2
Ciprofloxacin	97	58	59.8	149	143	96
Gentamycin	138	76	55.1	179	144	80.5
Imipenem	139	61	43.9	183	160	87.4
Meropenem	62	33	53.2	107	99	92.5
Piperacillin	89	39	43.8	120	119	99.2
Piperacillin/tazobactam	88	50	56.8	109	103	94.5

Table 3. Antibiotic resistance of *K. pneumoniae* and *E. coli* to antimicrobial agents

Antimicrobial agents	<i>K. pneumoniae</i>			<i>E. coli</i>		
	Total	N	%	Total	N	%
Amoxicillin/clavulanic acid	102	72	70.6	51	34	66.7
Ampicillin	96	96	100	52	43	82.7
Aztreonam	55	40	72.7	29	15	51.7
Cefepime	90	54	60	38	21	55.3
Cefotaxime	99	62	62.6	50	27	54
Ceftriaxone	55	31	56.4	28	13	46.4
Cefoxitin	68	30	44.1	40	11	27.5
Ceftazidime	99	63	63.6	49	26	53.1
Cefuroxime	99	61	61.6	50	28	56
Cephalothin	46	36	78.3	32	29	90.6
Ciprofloxacin	92	49	53.3	40	23	57.5
Gentamycin	106	54	50.9	51	24	47.1
Imipenem	106	14	13.2	50	3	6
Meropenem	42	7	16.6	27	1	3.7
Nitrofurantoin	32	24	75	28	9	32.1
Norfloxacin	30	13	43.3	27	12	44.4
Piperacillin	45	38	84.4	22	20	90.9
Piperacillin/tazobactam	85	40	47.1	44	21	47.4
Tetracyclin	56	34	60.7	30	21	70
Trimethoprim/sulfamethoxazole	104	67	64.4	50	36	72

Table 4. ESBL types among *E. coli*- and *K. pneumoniae*-ESBL producing isolates.

Isolated organisms (N)	TEM		SHV		CTX-M	
	N.	%	N.	%	N.	%
<i>K. pneumoniae</i> (43)	22	51.2	20	46.5	35	81.4
<i>E. coli</i> (17)	10	58.8	4	23.5	10	58.8

Table 5. Types of MBL in *P. aeruginosa* and *A. baumannii* clinical isolates

Isolated organisms (N)	<i>bla</i> VIM		<i>bla</i> IMP		<i>bla</i> VIM and <i>bla</i> IMP	
	N	%	N	%	N	%
<i>P. aeruginosa</i> (31)	6	19.4	7	22.6	2	6.5
<i>A. baumannii</i> (131)	22	16.8	26	19.8	6	4.6

4. Discussion

The current study's location was selected for its importance and relevance, since millions of Muslims travel to Makkah annually to perform Umrah and/or Hajj rituals. A mass gathering of so many people from different parts of the world in a limited area increases the susceptibility for infection [18]. In the present study, most patients infected by gram-negative bacteria were aged 60 years or above. The mean age of patients with infections in ICUs is known to be ~ 55.3 years [19]. The most common bacteria isolated in this study were *A. baumannii* followed by *P. aeruginosa*, *K. pneumoniae* and *E. coli*. In another local study, it was shown that *A. baumannii* remains the most common bacteria isolated from the ICU, followed by *P. aeruginosa*, *E. coli* and *K. pneumoniae* [20]. The majority of *P. aeruginosa* and *A. baumannii* were isolated from the Al-Noor Specialist Hospital. This hospital is the largest hospital in Makkah with approximately 560 beds and includes many clinical wards including burns department. It has been reported that infection is one of the most serious complications in burn patients, with *P. aeruginosa* being the most important, resistant and dangerous organism in infections in burns patients [21]. However, *K. pneumoniae* were most frequently isolated from specimens from Hera General Hospital. Further studies are needed to investigate the cause of increased *K. pneumoniae* infections in this hospital.

In the current study, the maximum number of *E. coli* isolates were found in urine specimens (43%), followed by wound swabs (17%). In a related study, 54% of isolated *E. coli* have been observed in urine specimens [22]. Moreover, *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* were the most pathogenic bacteria causing RTIs representing 77.5%, 67.6% and 49.1%, respectively. A study in China has previously demonstrated similar results, i.e. the most common pathogenic bacteria causing RTI were *A. baumannii*, *P. aeruginosa*, and *K. Pneumonia* [22]. *E. coli* was the causative organism most frequently causing UTIs in the present study, with identical findings being observed by the study in China [22].

P. aeruginosa and *A. baumannii* showed high resistance towards most antibiotics tested in this study. A lower susceptibility of *P. aeruginosa* and *A. baumannii* towards various antibiotics has been shown in a recent study in other regions of Saudi Arabia [20]. The susceptibility of strains isolated between 2004–2009 were compared, and it was concluded that antibiotic susceptibility was significantly decreased in many organisms including *A. baumannii* and *P. Aeruginosa* [20]. The study showed that *E. coli* and *K. pneumoniae* isolates had a high susceptibility to imipenem, while a moderate susceptibility to gentamycin. Aljohani *et al.* [20] observed that antibiotic susceptibility markedly decreased in *E. coli* between 2004–2009, reaching 50% for cefuroxime, ceftazidime, cefotaxime and cefepime. Similarly, another international study has shown that *E. coli* and *K. pneumoniae* is highly susceptible to imipenem [22].

Both *bla*IMP and *bla*VIM genes have been reported worldwide in clinical isolates of gram-negative pathogens [23,24]. Many previous studies have reported that IMP- and VIM-producing *Pseudomonas* isolates are distributed worldwide [21,25]. The clinically important MBL families are located in horizontally transferrable gene cassettes and can be spread among gram-negative bacteria [25]. In the present study, 73.8% of *P. aeruginosa* isolates were identified as MBL-producing isolates, 48.4% of these isolates harbouring MBL genes; *bla*VIM and *bla*IMP were distributed in 19.4% and 22.6% of the isolates, respectively. Regarding *A. baumannii*, only 16.8% and 19.8% possessed the *bla*VIM and *bla*IMP genes, respectively. In contrast, a recent study in Saudi Arabia showed that all *P. aeruginosa* MBL-producing isolates harboured a VIM-like gene [26]. Similarly, *P. aeruginosa* strains isolated from a hospital in Iran possessed *bla*VIM gene, although no *bla*IMP gene was detected [25]. Studies reporting VIM are more numerous than those involving IMP; in some studies, IMP has not been detected in isolated MBL-producing *Pseudomonas* strains [27,28].

The results in the present study demonstrated that certain isolates presented positive results for MBL

production but did not harbour either *blaVIM* or *blaIMP* genes. This could be explained by the presence of putative proteins belonging to MBLs other than IMP- and VIM-predominant MBLs. Many MBL-producing isolates in this study were multidrug-resistant, which is a major problem in choosing antibiotic therapy. The multidrug resistance of these isolates plays an important role in the colonization or infection of chronically hospitalised patients [21]. For efficient treatment of nosocomial infections caused by such multi-resistant isolates, clinicians often have to choose the most effective fluoroquinolones or combinations of different antibiotics [21]. Over the last several decades, many β -lactamases have emerged due to the extensive use of β -lactam antibiotics in clinical practice.

Gram-negative bacilli can also produce ESBLs, which are enzymes that have the capability to hydrolyse β -lactam antibiotics containing an oxyimino group (third-generation cephalosporins and aztreonam); these are inhibited by β -lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam [29]. ESBLs are usually plasmid-mediated β -lactamases, most commonly found in *K. pneumoniae*, *E. coli* and other gram-negative bacilli [30]. ESBL enzymes are classified into nine families based on their amino acid sequences, wherein TEM, SHV and CTX-M enzymes form major families [30]. In the current study, 170 clinical isolates (*K. pneumoniae*, 116; *E. coli*, 54) were isolated from ICUs and assessed for ESBL production based on CLSI guidelines. Of these, 60 isolates (*K. pneumoniae*, 37.1%; *E. coli*, 31.5%) were identified as ESBL-producing strains. A previous studies in Saudi Arabia have demonstrated a moderate to high rate of ESBL production (24.4% - 55%) by *K. pneumoniae* [31]. In contrast, another Saudi Arabian study has reported a low rate of ESBL production in *K. pneumoniae* (7.5–10.4%) and *E. coli* (8%) in the eastern region [32,33]. In India, a high prevalence of ESBL production has been reported, ranging from 41.0% to 63.6% in *E. coli* and from 40% to 83.3% in *K. pneumoniae* [34-36]. The present results reveal a lower incidence of ESBL production than that reported in Turkey, India and Korea for *K. pneumoniae* [34,37,38]. However, the antibiotic resistance among *E. coli* isolates in the present study was similar or higher than that reported in other countries [37,38]. The most common β -lactamase type presented in this study was CTX-M, followed by TEM and SHV in *K. pneumoniae*, while *E. coli* isolates showed an equal percentage of strains harbouring TEM and CTX-M (58.8%) with a low frequency for SHV β -lactamase genes. These results support the hypothesis that CTX-M is emerging as the dominant ESBL type in clinical isolates [29].

Conclusion

The present study thus highlights the high rates of antibiotic resistance in *P. aeruginosa*, *A. Baumannii*, *E. coli* and *K. pneumoniae*. More intensive infection control measures are required to prevent the further spread of resistant strains. Several ESBL and MBL types exist among clinical isolates in the Makkah region, indicating the importance of accurate and timely laboratory detection of ESBL- and MBL-producing isolates for optimal treatment of patients and for controlling the nosocomial spread of such strains. Continuous monitoring of antimicrobial susceptibility is recommended for reducing antibiotic resistance in the future.

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