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ORIGINAL ARTICLE

Healthcare Associated Infections Caused by Gram-negative Bacilli in Adult Intensive Care Units: Identification of AmpC Beta-Lactamases Mediated Antimicrobial Resistance

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ABSTRACT

Background: *Healthcare associated infections (HAIs) caused by AmpC β-lactamases producers are clinically significant as they can mediate resistance to cephalosporins, penicillins, monobactams and cephamycins.* ***Objectives:*** *To find out the prevalence of AmpC producers among Gram-negative bacilli recovered from HAIs in adult intensive care units (ICUs) and to identify the risk factors associated with AmpC β-lactamases production. In addition, we compared the antimicrobial resistance patterns in AmpC positive and negative isolates.* ***Methodology:*** *A prospective study was conducted over one year duration on samples collected from patients suffering from HAIs in the adult ICUs of Mansoura Emergency Hospital, Mansoura- Egypt. Isolated Gram-negative bacilli were subjected to cefoxitin disk diffusion test to screen for AmpC production. Presumptive AmpC producers were then confirmed by AmpC disk test, disk approximation test and AmpC E test.* ***Results:*** *Out of the recovered 240 Gram-negative bacilli, 73 isolates (30.4%) were AmpC producers. Acinetobacter baumannii represented the highest AmpC production (44.4%). Significant risk factors for AmpC β-lactamases production included previous exposure to antibiotic therapy, prolonged duration of antibiotic therapy before development of HAIs and presence of a central line or endotracheal tube. Resistance to all tested antimicrobials, with the exclusion of imipenem and meropenem, was significantly associated with AmpC producers.* ***Conclusion****: Increased prevalence of AmpC producers among Gram-negative isolates was observed in our study. Thus, early detection of AmpC producing bacteria is necessary to avoid treatment failure. Establishment of antibiotic stewardship policy and enhanced infection prevention and control measures are necessary to avoid spread of resistant infections in ICUs.*

INTRODUCTION

Healthcare associated infections (HAIs) are infections linked to the different aspects of medical care delivered in healthcare settings. Such infections are clinically important as they lead to increased morbidity and mortality along with the medical care expenses. Patients in high risk areas, as intensive care units (ICUs), are highly predisposed to HAIs due to their acute clinical conditions along with their frequent exposure to invasive procedures. In addition, indiscriminate use of antimicrobial agents in ICUs has led to increase in the HAIs caused by multi-drug resistant organisms[1,2].

Gram-negative bacilli are considered to be common etiological agents of HAIs encountered in ICUs. Treating such infections can be challenging because of frequently encountered antimicrobial resistance among these isolates [3]. Multi-drug resistant isolates of Gram-negative bacilli have increased over the past decades because of their ability to produce various types of β-lactamases, in particularly, extended spectrum β-lactamases (ESBL) and AmpC β-lactamases. Beta-lactamases are known to hydrolyze and deactivate β-lactam class of antibiotics, thus mediating resistance to this antimicrobial class [4]. These enzymes are clinically significant as they narrow antibiotic choices and result in treatment failure, besides, they are increasingly reported worldwide [1].

AmpC β-lactamases are cephalosporinases, that belongs to class C β-lactamases as per Ambler structural classification in 1980[5] and group I according to the functional classification by Bush and his colleagues [6]. AmpC β-lactamases mediate resistance to several β-

lactams as penicillins, cephalosporins and monobactams. In contrast to ESBL, AmpC β-lactamases are also able to hydrolyze cephamycins as cefoxitin and cefotetan. Furthermore, AmpC β-lactamases producers can resist the action β-lactam/ β-lactamase inhibitor combinations as ampicillin/clavulanic acid and piperacillin/tazobactam [7,8]. Unlike the ESBL which are inhibited by clavulanic acid, AmpC enzymes can be inhibited by boronic acid and cloxacillin [9]. Although infections caused by AmpC producers carry high clinical significance, the Clinical Laboratory Standards Institute (CLSI) has not yet released guidelines for their identification [1,3].

Chromosomal AmpC β-lactamases encoding genes are frequently present in Gram-negative bacilli [10]. The expression of chromosomal *ampC* genes is triggered by β-lactam antibiotics, including cefotetan, cefoxitin and imipenem[9]. In addition to the chromosomal *ampC* genes, the prevalence of plasmid-encoded AmpC β-lactamases has been increasingly reported [11,12]. Plasmid AmpC β-lactamases are frequently associated with resistance to quinolones, aminoglycosides and cotrimoxazole. Furthermore, porin mutations in plasmid mediated AmpC producers can lead to carbapenems resistance [13,14].

Infections caused by AmpC β-lactamases producers can constitute a clinical challenge to treating physicians. Detection of AmpC β-lactamases producers may play a significant part in creating proper antibiotic policy and thus, helping the clinicians to choose the proper treatment [3]. Therefore, it is essential to identify the true prevalence of AmpC producers especially in high risk areas as ICUs where the infections caused by resistant pathogens are higher [1]. In this study, we determined the prevalence of AmpC producers among Gram-negative bacilli recovered from HAIs in adult ICUs. In addition, we investigated the risk factors for AmpC β-lactamases production and compared the antibiotic resistance patterns in AmpC positive and negative isolates.

METHODOLOGY

Setting:

This prospective study was conducted over one year duration, from April 2016 till March 2017, at the Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University. The institutional review board and the ethical committee has revised and approved this study.

Selection of Patients and Sample Collection:

The study participants included patients who developed HAIs during their stay in the adult ICUs of Mansoura Emergency Hospital, Mansoura- Egypt. Various types of HAIs were identified as per the guidelines of the Centers for Disease Control and Prevention (CDC) [15]. According to the CDC definitions, HAIs were identified when the event date; the first element of infection, occurred on or after the third calendar day of hospital stay with the day of admission considered as the first calendar day.

Possible risk factors were documented for all the study subjects including age, gender, existence of indwelling invasive devices as endotracheal tubes and central lines, duration of ICU stay before the development of HAIs, duration of antibiotic therapy before HAIs and previous exposure to antimicrobials. Previous exposure to antimicrobials was defined as antimicrobial use within 14 days prior to the HAI [16].

Different clinical samples were collected including blood, urine, endotracheal aspirates and surgical wound drainages using aseptic technique from patients having HAIs. Collected samples were immediately sent to the laboratory for processing. Written consent was taken from each participant or his legal guardian.

Sample Processing and Identification of Gram-negative Bacilli:

All samples were processed according to standard microbiological techniques. Gram-negative bacilli were identified by Gram-stained films, colonial morphology and biochemical reactions of the isolates. Besides, API 20E and API 20NE identification kits (bioMerieux-France) were used to confirm the initial identification of Gram-negative bacilli.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibilities of all recovered Gram-negative bacilli were tested by disk diffusion method using Mueller-Hinton agar (MHA) and the following antibiotic disks (Oxoid- UK): gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), cefepime ((30 µg), aztreonam (30 µg), piperacillin (100µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg) and meropenem (10 µg). The procedure of disk diffusion test along with the reading of the results was performed in accordance with the guidelines of CLSI [17].

Screening Test for AmpC β-Lactamases Production:

All the isolated Gram-negative bacilli were tested for AmpC β-lactamases production by using cefoxitin disk diffusion test. Standard disk diffusion test procedure was conducted using MHA plates and 30 µg-cefoxitin disks (Oxoid- UK). After incubation of the MHA plates, the inhibition zones of the isolates were measured. Isolates resulting in inhibition zones with diameter smaller than 18 mm were considered as presumptive AmpC β-lactamases producers [3]. Presumptive AmpC β-lactamases producers were further subjected to AmpC production confirmatory tests that included AmpC disk test, disk approximation test and AmpC E test.

Confirmatory Tests for AmpC β-Lactamases Production:

AmpC disk test

AmpC disk test was first developed by Black et al., in order to detect the production of AmpC β-lactamases [18]. In this test, Tris–EDTA was utilized because of its

ability to increase the permeability of bacterial cells with subsequent release of β-lactamases. Preparation of AmpC disks was conducted in the laboratory by using sterile filter paper disks and one to one mixture of 100X Tris-EDTA (Fisher Scientific- UK) and saline. In order to prepare the AmpC disks, 20 µl of the prepared mixture were added to the filter paper disks. After drying, the prepared AmpC disks were stored at 2-8°C.

Cefoxitin susceptible *Escherichia coli (E. coli)* ATCC 25922 was used to inoculate the surface of MHA in accordance with standard disk diffusion procedure. Just before the procedure, saline (20 µl) was used to rehydrate the AmpC disks and several colonies of each presumptive AmpC β-lactamases producer were applied to the rehydrated disk. A cefoxitin disk (30 µg) was placed on the inoculated MHA plate. The AmpC disk, inoculated with presumptive AmpC producer, was then positioned nearly touching the cefoxitin disk with the inoculated face of the disc contacting the MHA surface. Then, the MHA plate was inverted and incubated aerobically overnight at 35 °C. Positive result for AmpC β-lactamases production was reported if the inhibition zone is flattened or indented because of cefoxitin inactivation. Lack of distortion of inhibition zone indicated absence of enzymatic inactivation of cefoxitin and was reported as negative result for AmpC production [18]. Cefoxitin sensitive *E. coli* ATCC 25922 was used as a negative control for the test.

Disk approximation test

In order to execute the disk approximation test, standard disk diffusion method was performed. A ceftazidime disk (30 µg), the reporter substrate, was located at the center of the inoculated MHA plate. Three antibiotic disks; cefoxitin (30 µg), amoxicillin-clavulanate (20/10 µg) and imipenem (10 µg) were used as inducers and positioned with 2 cm distance from the centered ceftazidime disk. After overnight incubation, positive result for AmpC β-lactamases production was considered if there is any flattening or blunting of the inhibition zone between the ceftazidime disk and the inducer disks [8].

AmpC E test

Presumptive AmpC β-lactamases producers were also subjected to AmpC E test (bioMerieux- France). The E test AmpC strip contains cefotetan (CN) at one end while the other end contains cefotetan-cloxacillin (CNI). The test was performed as stated in the manufacturer's instructions. If the MIC ratio of CN/CNI ≥8, a positive result for AmpC production was reported.

Statistical Analysis

Student T test was used to compare parametric data that was presented as mean ±SD while non-parametric data were compared using the Mann-Whitney U test. Statistical analysis was performed using SPSS 22.0 for Windows XP OS (SPSS Inc., Chicago, IL, USA). Statistical significance was considered when P value < 0.05.

RESULTS

Over the duration of this study, 240 Gram-negative bacilli strains were isolated from different samples that were collected from patients developing HAIs in adult ICUs. Of the recovered isolates, *Pseudomonas aeruginosa (P. aeruginosa)* was the commonest (31.3%), followed by *E. coli* (25.8%), *Klebsiella pneumoniae (K. pneumoniae)* (19.2%), *Acinetobacter baumannii (A. baumannii)* (18.8%) *and Proteus mirabilis (P. mirabilis)* (5%). Out of the 240 Gram-negative bacilli, 85 (35.4%) were isolated from urine, 78 (32.5%) from endotracheal aspirates, 56 (23.3%) from blood and 21 (8.8%) from surgical wound drainages. *Escherichia coli* was the predominant isolate recovered from the urine samples (45.9%) while *P. aeruginosa* and *A. baumannii* were the commonest isolates from endotracheal secretions and blood respectively. Table 1 demonstrated the distribution of Gram-negative bacilli isolates in relation to different samples.

Table 1. Distribution of isolated Gram-negative bacilli according to the source

Isolates	Sample				Total
	Urine	Endotracheal aspirates	Blood	Wound drainages	
P. aeruginosa	17 (20.0)	35 (44.9)	17 (30.4)	6 (28.6)	75 (31.3)
E. coli	39 (45.9)	7 (8.9)	11 (19.6)	5 (23.8)	62 (25.8)
K. pneumoniae	5 (5.9)	28 (35.9)	9 (16.1)	4 (19.0)	46 (19.2)
A. baumannii	14 (16.5)	8 (10.3)	18 (32.1)	5 (23.8)	45 (18.8)
P. mirabilis	10 (11.8)	0	1 (1.8)	1 (4.8)	12 (5.0)
Total	85 (100)	78 (100)	56 (100)	21 (100)	240 (100)

P. aeruginosa=Pseudomonas aeruginosa, E. coli =Escherichia coli, K. pneumoniae=Klebsiella pneumoniae
A. baumannii=Acinetobacter baumannii, P. mirabilis=Proteus mirabilis
Values are expressed as n (%)

All recovered 240 Gram-negative isolates were subjected to cefoxitin disk diffusion test in order to screen for AmpC β-lactamases production. Based on the results of cefoxitin disk diffusion test, 149 isolates were identified as presumptive AmpC producers. Among the 149 presumptive AmpC producers, 73 (49.0%) showed positive results for AmpC β-lactamases production by confirmatory tests.

In the present study, AmpC production was identified in 73 out of 240 (30.4%) isolated Gram-negative bacilli. The 73 AmpC positive isolates included 22 *E. coli* (30.1%), 20 *A. baumannii* (27.4%), 14 *P. aeruginosa* (19.2%), 14 *K. pneumoniae* (19.2%) and 3 *P. mirabilis* (4.1%) as revealed in table 2.

Table 2: Distribution of AmpC β-lactamases producers according to the source

AmpC β-lactamases producers	Sample				Total
	Urine	Endotracheal aspirates	Blood	Wound drainages	
P. aeruginosa	1 (6.7)	12 (32.4)	1 (6.3)	0	14 (19.2)
E. coli	10 (66.7)	6 (16.2)	5 (31.2)	1 (20.0)	22 (30.1)
K. pneumoniae	1 (6.7)	11 (29.7)	1 (6.3)	1 (20.0)	14 (19.2)
A. baumannii	2 (13.3)	8 (21.6)	8 (50.0)	2 (40.0)	20 (27.4)
P. mirabilis	1 (6.7)	0	1 (6.3)	1 (20.0)	3 (4.1)
Total	15 (100)	37 (100)	16 (100)	5 (100)	73 (100)

P. aeruginosa=Pseudomonas aeruginosa, E. coli =Escherichia coli, K. pneumoniae=Klebsiella pneumoniae

A. baumannii=Acinetobacter baumannii, P. mirabilis=Proteus mirabilis
Values are expressed as n (%)

Highest AmpC production was identified among isolated *A. baumannii* as 44.4% of the recovered isolates were AmpC producers, followed by *E. coli* (35.5%), *K. pneumoniae* (30.4%), *P. mirabilis* (25.0%) and *P. aeruginosa* (18.7%) as shown in table 3. Of the 73 AmpC positive isolates in this study, 37 (50.7%) were recovered from endotracheal aspirates, 16 (21.9%) from blood, 15 (20.5%) from urine and 5 (6.8%) from wound drainages. The prevalence of AmpC producers in different samples was demonstrated in table 4.

Table 3: Prevalence of AmpC β-lactamases producers among various Gram-negative bacilli

Isolates	Total number	AmpC β-lactamases producers n (%)
P. aeruginosa	75	14 (18.7%)
E. coli	62	22 (35.5%)
K. pneumoniae	46	14 (30.4%)
A. baumannii	45	20 (44.4%)
P. mirabilis	12	3 (25.0%)
Total	240	73 (30.4%)

P. aeruginosa=Pseudomonas aeruginosa,
E. coli = Escherichia coli,
K. pneumoniae=Klebsiella pneumoniae
A. baumannii=Acinetobacter baumannii,
P. mirabilis=Proteus mirabilis

Table 4: Prevalence of AmpC β-lactamases producers among various clinical samples

Sample	Total number of isolates	AmpC β-lactamases producers n (%)
Urine	85	15 (17.6%)
Endotracheal aspirates	78	37 (47.4%)
Blood	56	16 (28.6%)
Wound drainages	21	5 (23.8%)
Total	240	73 (30.4%)

In the present study, we investigated possible risk factors for AmpC β-lactamases production as presented in table 5. Among the analyzed risk factors, previous exposure to antibiotic therapy, prolonged duration of antibiotic therapy before the development of HAIs and presence of a central line or endotracheal tube were significantly associated with AmpC β-lactamases production. We also compared the antibiotic resistance patterns of AmpC β-lactamases positive and negative isolates as shown in table 6. With the exception of imipenem and meropenem, AmpC β-lactamases positive isolates were significantly associated with resistance to the tested antimicrobials.

Table 5: Distribution of risk factors in AmpC β-lactamases positive and negative isolates

Risk factors	AmpC β-lactamases positive isolates (n= 73)	AmpC β-lactamases negative isolates (n=167)	P Value
Age, mean±SD	63±3.2	64±2.5	0.92
Male gender, n (%)	35 (47.9)	82 (49.1)	0.83
Days of ICU stay before the development of HAIs, mean±SD	16±1.2	14±0.9	0.28
Previous exposure to antibiotic therapy, n (%)	68 (93.2)	82 (49.1)	0.02*
Days of antibiotic therapy before HAIs, mean±SD	8.3±1.7	5.2±0.7	0.01*
Presence of an indwelling invasive device			
Central line, n (%)	70 (95.9)	112 (67.1)	0.027*
Endotracheal tube, n (%)	68 (93.2)	109 (65.3)	0.034*

HAIs= Healthcare associated infections, ICU=Intensive care unit

Table 6: Antimicrobial resistance patterns in AmpC β-lactamases positive and negative isolates

Antimicrobials	AmpC β-lactamases positive isolates n=73	AmpC β-lactamases negative isolates n=167	P Value
Gentamicin	59 (80.8)	76 (45.5)	0.038*
Amikacin	55 (75.3)	59 (35.3)	0.04*
Ciprofloxacin	65 (89.0)	103 (61.7)	0.048*
Ceftazidime	73 (100)	105 (62.9)	0.035*
Cefepime	52 (71.2)	55 (32.9)	0.04*
Aztreonam	70 (95.9)	114 (68.3)	0.045*
Piperacillin	71 (97.3)	120 (71.9)	0.045*
Piperacillin-Tazobactam	63 (86.3)	57 (34.1)	0.02*
Imipenem	10 (13.7)	21 (12.6)	0.85
Meropenem	6 (8.2)	13 (7.8)	0.67

Values are expressed as n (%)

DISCUSSION

Healthcare associated infections caused by drug resistant Gram-negative bacilli that can produce various types of β-lactamases are increasingly reported in the ICUs with subsequent rise in morbidity, mortality and medical care expenses[1]. A major concern in treating the HAIs caused by Gram-negative pathogens is that antibiotic choices are running out[19]. AmpC β-lactamases enzymes are clinically significant due to their ability to mediate resistance to cephalosporins, aztreonam and β-lactam/ β-lactamase inhibitor combinations [20]. If not detected on time, AmpC producers can result in failure of treatment [21].

In the current study, 240 Gram-negative bacilli were recovered from HAIs in adult ICUs. The commonest Gram-negative bacilli were *P. aeruginosa* (31.3%) and *E. coli* (25.8%). In agreement with our results, Dasgupta and his colleagues[22] reported that *P. aeruginosa* and *E. coli* were the commonest etiological agents of HAIs in ICUs. Urinary tract infection (UTI) was the most frequently identified type of HAIs in our study (35.4%), followed by pneumonia (32.5%), bloodstream infection (23.3%) and surgical site infection (8.8%). Previous studies reported that UTI was the commonest HAI encountered in ICUs which was in line with our results[1,22].

Screening of Gram-negative isolates for AmpC β-lactamases production was conducted by cefoxitin disk diffusion test and resulted in 149 presumptive AmpC producers. Of those 149 presumptive AmpC producers, 73 (49.0%) were confirmed as AmpC producers by confirmatory tests. Parallel to our results, Madhavan et al.[23] reported that 51% of potential AmpC producers showed AmpC production by confirmatory tests.

In the present study, we detected AmpC production in 73 out of 240 isolated Gram-negative bacilli with a prevalence of 30.4% which was disturbingly high in comparison to other studies. Oberoi and his colleagues[1] reported that the prevalence of AmpC producing isolates among Gram-negative bacilli recovered from ICUs was 5.4% which was significantly lower than our results. In another study by Kaur et al.[3] 13.3 % of Gram-negative bacilli were identified as AmpC producers. The prevalence of AmpC producers was reported to be 17.3% in Kolkata [7] and 22.9% among Gram-negative bacilli isolated from burn patients [24].

The high prevalence of AmpC producers reported in our study might be attributed to different geographical locations. It has been found that prevalence of β-lactamases varies in different countries and even among different settings in the same country [1]. These variations in β-lactamases prevalence could explain the differences in antimicrobial resistance patterns among various settings. High prevalence of AmpC producers among our isolates could also be attributed to indiscriminate use of antibiotics that can induce the expression of the AmpC β-lactamases encoding genes. Furthermore, lack of proper infection control measures could facilitate the spread of these genes between patients with subsequent rise in the prevalence of AmpC producers. In developing countries, including Egypt, improper staff /patient ratio along with financial restrictions could lead to the lack of implementation of infection control precautions. Under these circumstances, resistant Gram-negative bacilli can disseminate from one patient to another leading to more infected cases.

In this study, *A. baumannii* represented the highest AmpC production as 44.4% of the recovered isolates were AmpC producers. In accordance with our results, Kaur et al.[3] and Goel et al.[25] found that the highest AmpC production was among *A. baumannii* isolates with a reported prevalence of 43.5% and 23.3% respectively. In contrast to our results, Subha and his colleagues[26] reported that maximum AmpC production was within *E.* coli isolates (37%).

Among the recovered *E. coli* isolates in our study, 35.5% were AmpC producers. Similar to our results, Tewari and his collaugues[27] reported that 34% of investigated *E. coli* isolates were AmpC producers. In another study, the prevalence of AmpC production within *E. coli* isolates was 6.5% which was considerably lower than our findings[28]. Kaur et al.[3] have similarly reported a low prevalence of 7.8% among *E. coli* isolates.

AmpC producers constituted 30.4% of *K. pneumoniae* isolates in our study which was higher than the prevalence reported by Kaur et al.[3] (14.4%) and Sheemar et al.[29] (17%). We found that 18.7% of *P. aeruginosa* isolates were AmpC producers which was close to the prevalence of 15.3% reported by Goel and his colleagues[25]. In another study, a lower prevalence of AmpC production among *P. aeruginosa* (2.5%) was reported[3].

The majority of AmpC positive isolates in our study were recovered from endotracheal aspirates (50.7%), followed by blood (21.9%), urine (20.5%) and wound drainages (6.8%). In accordance with our results, Kaur et al., reported that 56.8% and 22.1% of the AmpC producers were isolated from respiratory samples and blood respectively [3].

Among the investigated risk factors in our study, previous exposure to antibiotic therapy, prolonged duration of antibiotic therapy before the development of HAIs and presence of a central line or endotracheal tube were significantly associated with AmpC β-lactamases production. The presence of urinary catheters was not considered as a point of comparison between the two groups because all the study participants had urinary catheters. The expression of chromosomal *ampC* genes is induced by β-lactams [9]. Similarly, some plasmid *ampC* genes are inducible by β-lactam antibiotics [30]. Therefore, previous exposure to antibiotic therapy and prolonged duration of antibiotic therapy before the development of HAIs can act as inducing factors for the AmpC β-lactamases production which could explain our findings. Furthermore, they can result in selection of resistant bacterial strains. In agreement with our findings, Pascual and his colleagues[31] reported that indwelling external devices and previous antimicrobial exposure were significant risk factors for AmpC production. These results underline the importance of judicious usage of antimicrobials inside the ICUs.

The high prevalence of AmpC producers among Gram-negative bacilli may reflect that more isolates are acquiring different mechanisms of antimicrobial resistance posing a therapeutic challenge. In our study, resistance to all tested antimicrobials, with the exception of imipenem and meropenem, was significantly associated with AmpC producers. In accordance with our findings, previous studies reported that AmpC producers displayed a higher degree of resistance toward different antimicrobials [3,21,24,32]. Low resistance of AmpC producing isolates to imipenem and meropenem in our study emphasized the importance of carbapenems in treating infections caused by AmpC producers.

Different types of β-lactamases such as ESBL, metallo-β-lactamases and oxacillinases were previously reported in Egypt [33-36]. In the present study, we reported high prevalence of AmpC β-lactamases producers in the ICUs that underlined the importance of early detection of these organisms by simple laboratory methods. Early detection of AmpC producers can assist the treating doctors in choosing the proper antibiotic therapy for their patients. Furthermore, following isolation precautions for patients harboring these multi-drug resistant isolates can prevent their further spread. Other measures to prevent dissemination of resistant bacterial strains in ICUs include ongoing surveillance of resistant organisms and strict application of infection control measures.

AmpC β-lactamases production differs in various healthcare facilities with subsequent variations in antibiotic susceptibilities among these facilities. This highlights the need to find out the prevalence of AmpC producers in different settings in particular the high risk areas as ICUs. Using these data would help each individual setting to tailor its own antimicrobial stewardship policy according to local antibiotic resistance patterns combined with international

guidelines. This is essential to adjust antimicrobial usage, limit selective pressure, ensure effective treatment and decrease the side effects of antibiotics [37].

CONCLUSION

Healthcare associated infections caused by AmpC β-lactamases producers are clinically significant due to the associated treatment challenges. Therefore, it is important to identify these isolates especially in critical areas as ICUs. We reported a high prevalence of AmpC producers (30.4%) among Gram-negative bacilli recovered from HAIs in adult ICUs. *Acinetobacter baumannii* presented the highest AmpC production followed by *E. coli*. Significant risk factors for AmpC β-lactamases production included previous exposure to antibiotic therapy, prolonged duration of antibiotic therapy before the development of HAIs and the presence of a central line or endotracheal tube. Resistance to all tested antimicrobials, with the exclusion of imipenem and meropenem, was significantly associated with AmpC producers. Thus, carrying out an antimicrobial stewardship policy established according to local sensitivity patterns and enhancement of infection prevention and control measures are needed to ensure proper antibiotic choice by the clinicians and to avoid spread of resistant infections in ICUs.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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