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ORIGINAL ARTICLE

Screening for Extended-spectrum β-lactamases, AmpC and Carbapenemases Producing Bacteria among Adult and Pediatric Intensive Care Unit Patients in Two University Hospitals in Egypt

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ABSTRACT

Background: Emerging β-lactam resistant gram-negative bacteria (BLR-GNB) is a problem worldwide, particularly in the Intensive Care Units (ICUs). **Objective:** The aim of the present work was to evaluate the rate of carriage and the acquisition rate of BLR-GNB at one adult and one pediatric ICU in Alexandria University Hospitals, Egypt. **Methodology:** One hundred adult and 50 pediatric intensive care unit patients were included in the study. Rectal and nasal swabs were inoculated onto chromID ESBL (BioMérieux). The rate of carriage and the acquisition rate of BLR-GNB were calculated in both ICUs. All identified GNB were tested for ESBL, AmpC-β lactamase and carbapenemase production using combined disc, AmpC disc, and modified Hodge test (MHT) respectively. **Results:** Eighty eight percent of adult and 74% of pediatric patients were BLR-GNB carriers. The acquisition rate of BLR-GNB in adult & pediatric ICUs 48 hrs after admission was 42.9% and 35% respectively. Out of the 150-screened patients, 280 BLR-GNB isolates were identified where *E. coli* was the most commonly isolated (45%) followed by *K. pneumoniae* (35%). **Conclusions:** The high rate of BLR-GNB carriage calls for routine surveillance, which will be helpful for monitoring the spread of drug resistance in ICU settings.

INTRODUCTION

Antimicrobial resistance is rapidly spreading allover the world and is a major public health threat. There have been the emergence and spread of genes coding for multi-drug resistance such as extended spectrum β-lactamases (ESBL), AmpC β-lactamases, and carbapenemases among *Enterobacteriaceae* and other Gram negative organisms. Many of these genes are plasmid mediated which has ensured their transfer and wide spread to many genera of bacteria.[1]

Intestinal flora form a major reservoir of GNB, all of which are potentially pathogenic for patients hospitalized in intensive care units (ICUs).[2] Gastrointestinal colonization by antibiotic resistant GNB is associated with subsequent clinical infection.[3]

Various risk factors for colonization by antibiotic resistant bacteria are identified such as previous hospitalization, nursing home residency, urinary catheter use, mechanical ventilation, previous antimicrobial use, old age, and comorbidities. The identification of carriers upon hospital admission is not only important for infection control measures but it is crucial, in case of severe infection, to treat patients with antibiotic therapy that is effective against antibiotic resistant bacteria.[4]

Different ESBL rates have been reported; for the Asia-Pacific region (27.7%), Latin America (23.3%), Europe (18.8%), the Middle East/Africa (16.2%), and North America (7.4%), which are much lower than those reported for India (≥ 80%) and China (≥ 60%).[5] Reports of carbapenem resistance have emerged during the past decade and are increasing rapidly.[6]

Universal screening for multi-drug resistant bacteria is time consuming, expensive and unlikely to be feasible.[7, 8] Targeted screening based on clinically based tools could therefore be useful. The development of such tools requires first to determine the local epidemiology of multi-drug resistant bacteria colonization in the endemic setting.[9]

Routine surveillance is deficient in Egyptian hospitals, and even if it is done it is targeting gram-positive bacteria and mainly methicillin resistant *S. aureus* (MRSA). In an attempt to evaluate the feasibility of introducing ICU admission BLR-GNB screening in Alexandria University hospitals, the present study was conducted to evaluate the rate of carriage and the acquisition rate of BLR-GNB in one adult and one pediatric ICU and to estimate the prevalence of ESBL, AmpC-β lactamase and carbapenemase producing isolates among the BLR-GNB isolates.

METHODLOGY

Study design and patient selection

One hundred patients from the main adult intensive care unit (MICU) at Alexandria Main University Hospital as well as 50 patients from pediatric intensive care unit (PICU) at Al-Shatby University pediatric Hospital were included in a prospective cross-sectional study. No exclusion criteria were adopted.

The acquisition rate (48 hrs after admission) was defined as the number of patients who were not colonized at admission but became colonized 48 hrs later, divided by the number of patients not colonized at admission.

Data collection

Demographic and clinical data concerning age, sex, clinical diagnosis, date of admission, associated infections, history of antibiotic intake, possible risk factors for colonization, outcome was collected from each patient.

Specimen collection and transport

Two surveillance swabs (one rectal and one nasal) were obtained from each patient. All patients were screened twice; rectal and nasal swabs were obtained on admission as well as 48 hours later. All swabs were put in Amies transport medium (Oxoid) and immediately transported to Microbiology Laboratory of Alexandria University Hospital to be processed.

Laboratory processing of samples

Inoculation and incubation of the culture media

All swabs were inoculated into sterile saline and vortexed for 5 seconds then 100 ul of resulting suspension was inoculated directly onto chromID ESBL (BioMérieux, Marcy l'Etoile, France). Plates were incubated at 35 °C ± 2 °C in ambient air and examined after 18-24 hours and 48 hours of incubation.[10]

Identification of isolated colonies

The color of each type of colony was recorded according to the chromID ESBL manufacturer's specifications. All colonies on chromID ESBL were picked for complete identification.

All colonies were regarded as presumptive cephalosporin resistant and/or beta lactamases producers (ESBL, &/or AmpC β-lactamase, &/or carbapenemase) and were identified to the species level by mass spectrometry MALDI-TOF MS UltraFlex system (Brüker Daltonik) according to the manufacturer's instructions.[11]

All identified organisms were further tested for ESBL, AmpC β-lactamase, and carbapenemases:

Initial screen tests:

A procedure similar to modified Kirby-Bauer for disc diffusion was performed using ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), cefepime (FEP, 30 µg) discs for screening for ESBL, cefoxitin (FOX, 30 µg) disc for screening of AmpC β-lactamase, and meropenem (MEM, 10 µg), imipenem (IPM, 10 µg) discs for screening for carbapenemases. The results were interpreted as per CLSI guidelines.[12]

Phenotypic confirmatory tests:

The isolates showing reduced susceptibility to CAZ (zone diameter of ≤ 22 mm) and CTX (zone diameter of ≤ 27 mm) were selected for screening of ESBL production. The isolates showing reduced susceptibility to cefoxitin (zone diameter of ≤ 18 mm) were selected for screening of AmpC enzyme production by AmpC disc test. The isolates showing reduced susceptibility to IPM or MEM (intermediately sensitive: 20-22 mm, resistant: <19 mm) were selected for screening of carbapenemases production by modified Hodge test.

ESBL confirmatory tests

a- ESBLs were detected by the CLSI confirmatory method using CTX (30 µg), CAZ (30 µg) alone and in combination with clavulanic acid (CLA).[12]

b- As cefepime was found to be a more reliable detection agent for ESBLs in the presence of an AmpC β-lactamase, FEP (30 µg) and FEP plus CLA; FEP/CLA (30/10 µg) test was also performed.[13]

A ≥ 5mm increase in the zone diameter of the cephalosporin alone and in combination with CLA was indicative of ESBL production.

Morphologic classification of ESBL to either the CTX-M or TEM/SHV types was made if it was detected by either CTX or CAZ respectively as previously described.[14]

AmpC disc test was performed as previously described.[15]

Modified Hodge test (MHT) was done as recommended by CLSI.[12]

The adopted algorithm for BLR-GNB screening and detection is shown in **figure 1**.

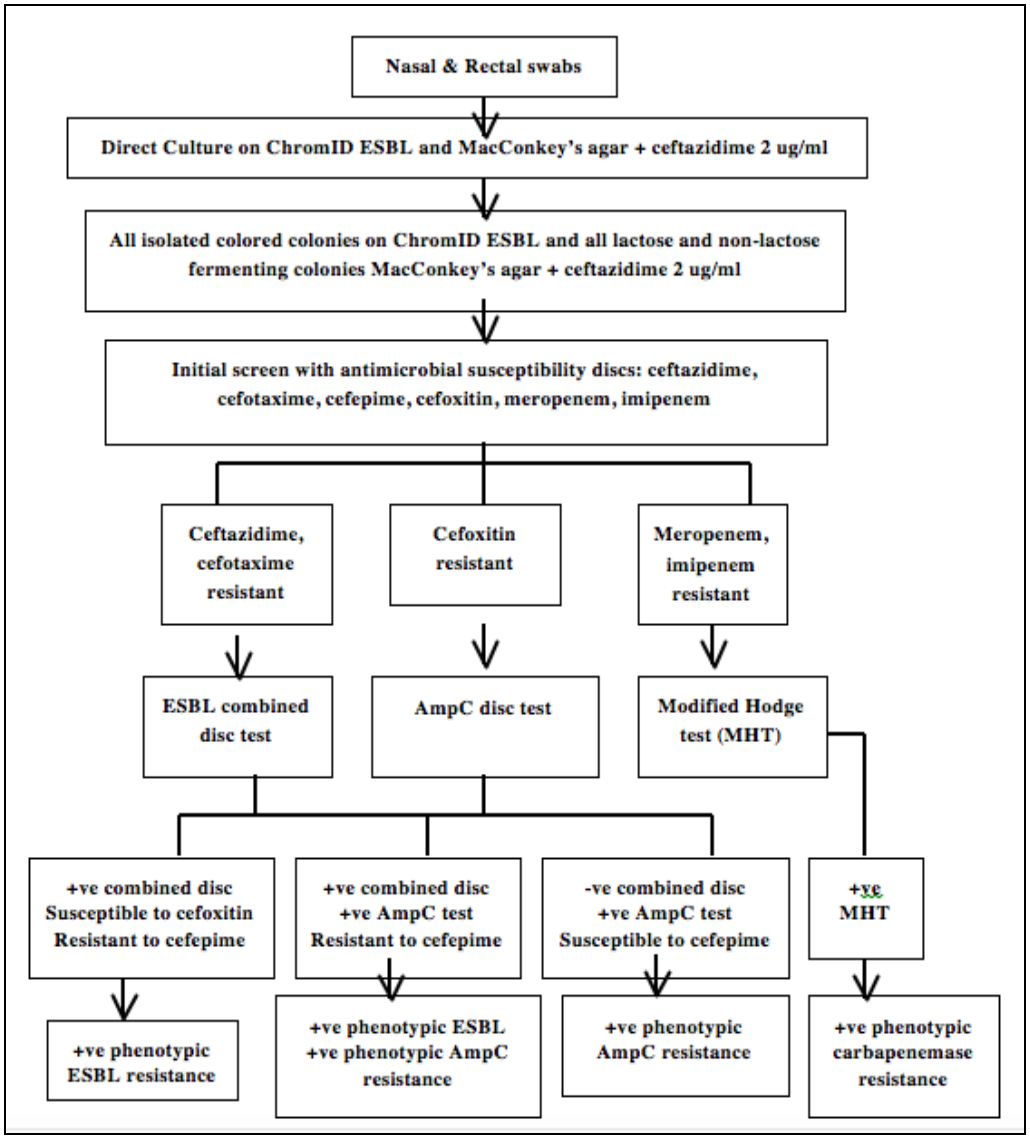

Fig 1. Algorithm for screening and phenotypic detection of ESBL, AmpC β-lactamase and carbapenemases in the present study.

Statistical analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc. Chicago, USA).

Ethical clearance was obtained from Alexandria University ethics Committee. An informed written consent was taken from the patients or their parents or guardians.

RESULTS

Demographic and clinical data results

The different demographic and clinical parameters of the 2 studied groups are shown in table 1.

Table 1. Demographic parameters of studied adult and pediatric ICU group.

Parameters	**MICU (n = 100)**	**PICU (n = 50)**
Age	45.5 (3 – 92 year)	8 (1 – 144 months)
Gender		
Male	52 (52%)	29 (58 %)
Female	48 (48%)	21 (42%)
Admission Diagnosis		
Trauma	21 (21%)	0 (0%)
Toxicology	11 (12%)	0 (0%)
Infectious	15 (15%)	26 (52.0%)
Respiratory	23 (23%)	15 (30.0%)
Cardiac	10 (10%)	7 (14.0%)
Hepatic	11 (11%)	0 (0.0%)
Endocrine	7 (7%)	5 (10.0%)
Renal	21 (21%)	2 (4.0%)
Neurological	4 (4%)	8 (16.0%)
Rheumatology	2 (2%)	0 (0.0%)
Gastrointestinal	0 (0%)	8 (16%)
Hematological	0 (0%)	4 (8%)
Severity of illness		
APACHE II score*	17.0 (8.0 – 29.0)	-
PIM 2 score^	-	60.0 (-31.0 – 100.0)
Mortality rate	33 (33%)	11 (22%)
Days of ICU stay	8.0 (0.0 – 32.0)	5.0 (2.0 – 18.0)
Type of infection		
Pneumonia	54 (54%)	5 (10%)
Urinary tract infection	20 (20%)	9 (18%)
Bloodstream infection	6 (6%)	4 (8%)
Others (Wound, Upper respiratory)	6 (6%)	8 (16%)
Organisms causing ICU infections		
Enterobacteriaceae	30 (30%)	3 (6%)
Gram negative non-fermenter bacilli	26 (26%)	0 (0%)
Gram positive cocci	15 (15%)	14 (28%)
Candida	9 (9%)	6 (12%)
Treating antibiotics		
Third generation cephalosporins	53 (53%)	17 (34%)
Fourth generation cephalosporins	24 (24%)	0 (0%)
Carbapenems	36 (36%)	44 (88%)
Anti-MRSA	26 (26%)	38 (76%)
Other antibiotics	56 (56%)	21 (42%)
Antifungal and antiviral	8 (8%)	14 (28%)

Patient may have more than one diagnosis, organisms, or given more than one antibiotic.
*Acute Physiology and Chronic Health Evaluation II (APACHE II) score.
^ Pediatric Index of Mortality 2 (PIM2) score.

Risk Factors for BLR-GNB Carriage

On admission, previous antibiotic use, previous hospitalization and hospital source of admission were found to be the identified risk factors for BLR-GNB carriage in MICU with adjusted odds ratio of 43.2, 8, and 7.8 respectively. Whereas the source of admission (from another department in the hospital) was the only identified risk factor for positive carriage in PICU with adjusted odds ratio of 95. The severity of illness as measured by APACHE II score was the only identified risk factor for BLR-GNB carriage after 48 hours of ICU admission in MICU with adjusted odds ratio of 1.3. Whereas, endotracheal intubation was the only identified risk factor for BLR-GNB carriage after 48 hours of PICU admission with adjusted odd ratio 4.7. (table 2 & 3)

Table 2. Analysis of risk factors associated with BLR-GNB colonization in adult patients.

	BLR-GNB carriage on admission					BLR-GNB carriage after 48hrs				
	Negative	**Positive**	**p value**	**OR (95%CI)**	**Adjusted OR (95%CI)**	**Negative**	**Positive**	**p value**	**OR (95%CI)**	**Adjusted OR (95%CI)**
MICU	**(n = 21)**	**(n = 79)**				**(n = 12)**	**(n = 9)**			
Age (months)	35 (5 – 65)	49 (3 – 92)	0.092	1 (0.9-1)		33 (5–60)	47 (22– 65)	0.081	1.05 (0.9-1.2)	1.1 (0.9-1.2)
Gender										
Male	11 (52.4%)	41 (51.9%)	0.969			7 (58.3%)	4 (44.4%)	0.670		
Female	10 (47.6%)	38 (48.1%)		1 (0.4-2.6)		5 (41.7%)	5 (55.6%)		1.8 (0.3-10)	
APACHE II score*	17 (12– 26)	18 (8 – 29)	0.137	1.1 (0.9-1.2)		14 (12 – 26)	19 (12 – 26)	**0.009**	**1.4 (1.0-1.8)**	**1.3 (1-1.8)**
Chronic Renal failure	1 (4.8%)	15 (19%)	0.181	4.7 (0.6-37)		0 (0.0%)	1 (11.1%)	0.429	-	-
Diabetes mellitus	1 (4.8%)	16 (20.3%)	0.113	5.1 (0.6-40)		0	1 (11.1%)	0.429	-	
Hypertension	2 (9.5%)	12 (15.2%)	0.728	1.7 (0.4-8)		2 (16.7%)	0 (0.0%)	0.486	-	
Liver cirrhosis	2 (9.5%)	9 (11.4%)	1.000	1.2 (0.2-6)		0	2 (22.2%)	0.171	-	
Chronic obstructive pulmonary disease	0	7 (8.9%)	0.340	-		0	0	-	-	
Central venous catheter	-	-	-	-		11 (91.7%)	9 (100.0%)	1.000	-	
Nasogastric tube	-	-	-	-		12 (100.0%)	8 (88.9%)	0.429	-	
Endotracheal intubation	-	-	-	-		10 (83.3%)	9 (100.0%)	0.486	-	
Previous Antibiotics	1 (4.8%)	62 (78.5%)	**<0.001**	**73 (9- 583)**	**43.2 (5-370)**	-	-	-	-	
Previous hospitalization	1 (4.8%)	23 (29.1%)	**0.020**	**8.2 (1-64)**	**8 (0.8-81)**	-	-	-	-	
Admission from the hospital	2 (9.5%)	52 (65.8%)	**<0.001**	**18.3 (4-84)**	**7.8 (1.4-45)**	-	-	-	-	
Antibiotic intake inside ICU	-	-	-	-		8 (66.7%)	8 (88.9%)	0.338	4 (0.36-44.1)	

*Acute Physiology and Chronic Health Evaluation II (APACHE II) score.

Table 3. Analysis of risk factors associated with BLR-GNB colonization in pediatric patients.

	BLR-GNB carriage on admission					BLR-GNB carriage after 48hrs				
	Negative	**Positive**	**p value**	**OR (95%CI)**	**Adjusted OR (95%CI)**	**Negative**	**Positive**	**p value**	**OR (95%CI)**	**Adjusted OR (95%CI)**
PICU	**(n = 20)**	**(n = 30)**				**(n = 13)**	**(n = 7)**			
Age (months)	5.5 (1 – 144)	10.5 (2 – 144)	0.131	1 (0.9-1)	1.1 (0.9-1.6)	4.0 (1 – 48)	12 (1 – 144)	0.202	1 (0.98-1.1)	
Gender										
Male	12 (60%)	17 (56.7%)	0.815			9 (69.2%)	3 (42.9%)	0.356		
Female	8 (40%)	13 (43.3%)		1.1 (0.4-3.6)		4 (30.8%)	4 (57.1%)		3 (0.44-20)	
PIM 2 score*	58.78 (5.8-100)	61.05 (-31-100)	0.729	1 (0.98-1.02)		45.3 (5.8 – 94)	65.6 (41 - 100)	0.068	1.03 (0.99 – 1.0)	
Hematological diseases	0 (0%)	4 (13.3%)	0.089	-		-	-	-	-	
Cardiac diseases	0 (0%)	4 (13.3%)	0.140	-		-	-	-	-	
Diabetes mellitus	3 (15%)	1 (3.3%)	0.289	0.2 (0.02-2)		-	-	-	-	
Nasogastric tube	0 (0 – 1)	0 (0– 1)	0.527	2.1 (0.2-21.8)		0 (0 – 1)	0 (0 – 1)	0.173	-	
Urinary catheter	20 (100%)	30 (100%)	-	-		13 (100.0%)	7 (100.0%)	-	-	
Central venous catheter	6 (30%)	11 (36.7%)	0.626	1.4 (0.4-4.5)		2 (15.4%)	4 (57.1%)	0.122	7.3 (0.8-61.3)	3.2 (0.3-38)
Endotracheal intubation	-	-	-	-		3 (23.1%)	5 (71.4%)	**0.062**	**8.3 (1-67.1)**	**4.7 (0.4-51)**
Previous Antibiotics	0	29 (96.7%)	**<0.001**	-		-	-	-	-	
Previous hospitalization	0	15 (50%)	**<0.001**	-		-	-	-	-	
Admission from the hospital	1 (5%)	25 (83.3%)	**<0.001**	**95 (10-882)**	**141 (13-1487)**	-	-	-	-	

*Pediatric Index of Mortality 2 (PIM2) score.

Carriage and acquisition rates results

Out of the 100 MICU patients, 79 patients (79%) screened positive, upon admission. However, 52 out of the 79 carriers were already transferred from another department in our hospital. Among the 79 carriers, 42 (53.2%) were rectal carriers only, eight (10.1%) were nasal carriers, and 29 (36.7%) were naso-rectal carriers of BLR-GNB. A total of 141 isolates were isolated on admission. Nine (out of 21) carrier negative MICU patients turned carrier-positive 48 hours after admission. Out of the nine carriers, three were rectal, one nasal and five naso-rectal carriers.

Concerning the PICU, Out of the 50 pediatric ICU patients, 30 patients (60%) were colonized with BLR-GNB on ICU admission, of which 25 were transferred to PICU from another site in the hospital. Out of the 30 patients, 27 (90%) were rectal carriers and three (10%) were naso-rectal carriers. A total of 38 resistant isolates were identified. Among the 20 initially non-colonized PICU patients, seven colonized patients were newly identified. Four patients were rectal carriers, two were naso-rectal and one was a nasal carrier. (Table 4)

Thus the acquisition rate of BLR-GNB in MICU 48 hrs after admission is 42.9% (9/21 patients). While, the acquisition rate in PICU is 35% (7/20 patients).

Table 4. Overview of BLR-GNB isolates and differences in species distribution in adult and pediatric ICUs.

Organism	Adult ICU		Pediatric ICU	
	On admission N (%)	> 48 hrs after admission N (%)	On admission N (%)	> 48 hrs after admission N (%)
E. coli **(n = 126)**	78 (55.4%)	18 (20.7%)	26 (68.4%)	4 (28.6%)
K. pneumoniae **(n = 98)**	47 (33.3%)	30 (34.5%)	11 (29%)	10 (71.4%)
A. baumanii **(n = 41)**	13 (9.2%)	27 (31%)	1 (2.6%)	0
P. mirabilis **(n = 6)**	2 (1.4%)	4 (4.6%)	0	0
C. freundi **(n = 4)**	0	4 (4.6%)	0	0
E. cloacae **(n = 4)**	0	4 (4.6%)	0	0
M. morganii **(n = 1)**	1 (0.7%)	0	0	0
Total (n = 280)	141	87	38	14

Results of screening and phenotypic tests

Out of the 150-screened patients, 280 BLR-GNB isolates were isolated. All isolates recovered on chromID ESBL gave the typical color according to the manufacturer's instructions, on the other hand, unexpectedly all *A. baumanii* isolated in the study gave white colored colonies on chromID ESBL agar which is originally designed by BioMérieux for isolation of *Enterobacteriaceae* only.

Among all isolated BLR-GNB, 94.6% (265/280) isolates were resistant to CTX, 63.9% (179/280) were resistant to CAZ, and 66.1% (185/280) were resistant to FEP. When the ESBL combined discs results were compared, it was observed that CTX-CLA detected the maximum number of isolates (159/171; 93%, 38/42; 90.5% in MICU and PICU respectively) while CAZ-CLA test only identified 100 (58.5%) MICU isolates and 14 (33.3%) PICU ESBL isolates.

Phenotypic evaluation of ESBL genetic mechanisms found that 58.5% (100/171) of MICU and 33.3% (14/42) of PICU isolates were detected by both CTX and CAZ which suggest CTX-M, SHV, or TEM-type ESBL. While, 34.5% (59/171) of MICU and 57.1% (24/42) of PICU isolates were detected with CTX alone suggesting CTX-M–type. Whereas none of the isolates were detected by CAZ/CLA combined disc alone.

Among the 213 ESBL positive isolates, 66 also tested positive for AmpC β-lactamase. Of those, 16 (24.2%) isolates were proved to be ESBL only by FEP/CLA combined disc test. Thus 16 isolates would have been missed by using only CLSI recommended method (Table 5).

As for screening for carbapenamase production, 23.2% (53/228) of MICU and 34.6% (18/52) of PICU isolates were resistant to MEM, while 21.5% (49/228) of MICU and 32.7% (17/52) of PICU isolates were resistant to IPM. Modified Hodge test confirmed carbapenemase production (CP) in 23.2% of MICU and in 34.6% of PICU isolates. Co-existence of different resistance mechanisms in MICU and PICU isolates is shown in **table 5**.

Finally, 88 out of 100 adult patients were BLR-GNB carriers distributed as follows: 88/88 (100%) ESBL carriers, 31/88 (35.2%) AmpC β-lactamase carriers, and 29/88 (33%) CP carriers. While, 37 out of 50 pediatric patients were BLR-GNB carriers, of which 37/37 (100%) were ESBL carriers, 8/37 (21.6%) were AmpC β-lactamase carriers, and 16/37 (43.2%) were CP carriers.

Table 5. Distribution of ESBL, AmpC β-lactamase, CP among BLR-GNB isolates in adult and pediatric ICUs.

Organism	ESBL	AmpC	CP	ESBL +AmpC	ESBL +CP	AmpC +CP	ESBL +Amp C +CP	Pure ESBL	Pure AmpC	Pure CP	Neg. by all tests
***E. coli* (n = 126)**											
MICU (n = 96)	87 (31%)	18 (6.4%)	7 (4.6%)	11 (3.9%)	0	0	7 (2.5%)	69 (24.6%)	0	0	9 (3.2%)
PICU (n = 30)	27 (9.6%)	3 (1.1%)	4 (1.4%)	0	0	0	4 (1.4%)	23 (8.2%)	0	0	3 (1.1%)
***K. pneumoniae* (n = 98)**											
MICU (n = 77)	62 22.1%	22 (7.9%)	25 (17.9%)	0	3 (1.1%)	0	22 (7.9%)	37 (13.2%)	0	0	15 (5.4%)
PICU (n = 21)	14 (5%)	5 (1.8%)	13 (4.6%)	0	8 (2.9%)	0	5 (1.8%)	1 (0.4%)	0	0	7 (2.5%)
***A. baumanii* (n = 41)**											
MICU (n = 40)	8 (2.9%)	16 (5.7%)	20 (14.3%)	0	0	8 (2.9%)	8 (2.9%)	0	0	12 (4.3%)	12 (4.3%)
PICU (n = 1)	1 (0.4%)	1 (0.4%)	1 (0.4%)	0	0	0	1 (0.4%)	0	0	0	0
***P. mirabilis* (n = 6)**											
MICU (n= 6)	6 (2.1%)	0	0	0	0	0	0	6 (2.1%)	0	0	0
PICU (n = 0)	0	0	0	0	0	0	0	0	0	0	0
***C. freundi* (n = 4)**											
MICU (n = 4)	4 (1.4%)	4 (1.4%)	1 (1.1%)	3 (1.1%)	0	0	1 (0.4%)	0	0	0	0
PICU (n = 0)	0	0	0	0	0	0	0	0	0	0	0
***E. cloacae* (n = 4)**											
MICU (n = 4)	4 (1.4%)	4 (1.4%)	0	4 (1.4%)	0	0	0	0	0	0	0
PICU (n = 0)	0	0	0	0	0	0	0	0	0	0	0
***M. morganii* (n = 1)**											
MICU (n = 1)	0	1 (0.4%)	0	0	0	0	0	0	1 (0.4%)	0	0
PICU (n = 0)	0	0	0	0	0	0	0	0	0	0	0
TOTAL (n = 280)	213 (76%)	74 (26.4%)	71 (25.4%)	18 (6.4%)	11 (3.9%)	8 (2.8%)	48 (17.1%)	136 (48.6%)	1 (0.4%)	12 (4.3%)	46 (16.4%)

DISCUSSION

Surveillance for BLR-GNB followed by the isolation of colonized patients has been thought to assist in control of the epidemic spread of ESBL- or AmpC-β-lactamase producing *Enterobacteriaceae*.[16, 17] Some authors have also called for routine surveillance to detect asymptomatic BLR-GNB colonization in an effort to control transmission.[18]

We reported high BLR-GNB carriage rate in our study which may call for the importance of active surveillance. These rates were higher than those reported for ESBL, AmpC β-lactamase or CP from different countries in Africa, Europe, Asia and America (8-42%).[19-23] The high rate of colonization among PICU patients may be due to early transmission from mothers especially through vaginal delivery. There is limited data on the carriage rate of community- acquired BLR-GNB in literature [21, 24, 25], in our study out of the 79 MICU carriers on-admission, 52 (65.8%) were transferred from another site in the hospital, thus only 27 (34.2%) can be considered community acquired. Similarly, out of the 30 PICU carriers, 25 were already hospitalized before ICU admission, thus only 5 (16.6%) were community carriers. Furthermore, the acquisition rate in our study (42.9% in MICU, 35% in PICU) even after only 48 hours of ICU admission was higher in comparison to previous studies.[20]

Concerning the risk factors for BLR-GNB carriage, previous antibiotic use, previous hospitalization and transfer from another department in the hospital were found to be risk factors for carriage in our study ($p<0.05$). This was confirmed in previous

studies.[26, 27] Of the various classes of antibiotics, third-generation cephalosporins had been the most incriminated in infection by ESBL-producing organisms.[26-28] This was also demonstrated in the present study. Our screening model was poorly predictive of risk factors for colonization of ICU patients. This may be attributed to the short duration of screening (only 48 hrs after admission and not until discharge) or the small number of patients included in the study.

In an attempt to decrease the workload for screening of carriage, we included the nasal swabs in our study, but according to our results they cannot replace the rectal swabs for screening of BLR-GNB carriage as the majority of identified carriers were rectally colonized (nasal carriers; 9/88 MICU and 1/37 PICU carriers).

The performance data of the evaluated ESBL-screening media (chromID ESBL) in this study is specifically based on detection of MDR organisms in general and not just ESBL-producing *Enterobacteriaceae*. Other studies concluded that chromogenic media appeared to have a significantly high sensitivity.[29] Of course, the main advantage of the chromID resided in its chromogenic character, which reduces the need for further identification. However, full identification of the isolate to spp. level and to subspp. level is absolutely necessary for surveillance purposes and evaluating infection control measures. MALDI-TOF is a rapid, accurate and inexpensive method for microbial identification. Its relative ease of use and rapid turnaround time makes it a powerful tool in the microbiology laboratory.[11]

In our study we selected the three cephalosporins; CAZ, CTX and FEP discs and combined with CLA for ESBL screening as the literature agreed that the use of CAZ and/or CTX alone as recommended by CLSI is not enough for detection of all ESBL isolates.[30] When combining the results of the 3 discs 100% of isolates of both ICUs were proved to be ESBL producers.

Phenotypic evaluation of ESBL genetic mechanisms suggested a relative predominance of the CTX-M type of ESBL which is in accordance with data recorded in Nigeria [26], and Thailand [31] but higher than that obtained in Birmingham, UK.[32] CTX-M ESBL genotype is tending towards a global pandemic.[26]

Out of the 109 MICU isolates, and the 24 PICU isolates showing resistance to cefoxitin in the present study, 74 (26.4%) were AmpC β-lactamase producers by AmpC test. This was in agreement with Yussif et al.[33], Singhal et al. [34], and also Sinha et al.[35] who reported AmpC enzyme production in 26.1%, 36% and 24% of the isolates respectively. Cefoxitin resistance in AmpC β-lactamase negative isolates could be due to a decreased permeability of porins or other resistance mechanisms.[33]

Cefepime is a more reliable detection agent for ESBLs in the presence of an AmpC β-lactamase, as this drug is stable to AmpC β-lactamase and will thus demonstrate the synergy arising from the inhibition of ESBL by clavulanate in the presence of AmpC enzyme.[1] Cefepime in double-disk synergy tests was first used for the detection of ESBLs among AmpC β-lactamase producers by Tzelepi et al.[13] Out of the 18 isolates (6.4%) co-producers of ESBL and AmpC β-lactamase in the present study, 16 (88.9%) were ESBL positive by FEP/CLA test only.

Some authors have proposed the use of chromogenic media for ESBL-producing isolates for detection of carbapenemase fecal carriers.[36, 37] These ESBL-chromogenic media lack specificity for CP, increasing the need for laboratory confirmation tests to discriminate CP from ESBL isolates. However, variations in carbapenem susceptibility of CP support the importance of basing the screening on ceftazidime rather than on a carbapenem.[38] This was the reason of our trial to use the same ChromID ESBL medium for screening of all types of β-lactam resistance in the study.

The very high rate (25.4%) of carbapenemase production confirmed by MHT in this study is alarming. This rate is higher than that reported in Spanish and French hospitals.[38, 39] In hospitals where carbapenemase-producing bacteria are becoming endemic, they might be selected under carbapenem exposure [2], and this was most probably the case in our hospitals.

Co-production of ESBL, AmpC β-lactamase and carbapenemase was noticed in the present study in 17.1% of the isolates as some of the isolates produce different combinations of the enzymes. Similar co-production of enzymes has been reported; Sinha et al.[35] reported 8% co-existence in E. coli. This co-production is indicative of the occurrence of multi-drug resistant pathogens, and possible associated aminoglycosides trimethoprin sulfamethotazole, fluroquinolone resistance because many bacteria carrying genes for AmpC β-lactamase, ESBL or carbapenemases on their plasmids may also carry genes responsible for resistance to other non β-lactam antibiotics.[33]

The present study has some limitations. First, it was not possible to perform molecular characterization of the enzymes expressed by the bacteria. Second, The study was conducted amongst high-risk ICU patients and thus our findings might not be generalizable to other settings and patient populations. Third, our failure to identify a predictive risk profile from available clinical data highlights the difficulties in implementing targeted on-admission screening programs.

CONCLUSION

Our study provides valuable information on the prevalence and epidemiology of BLR-GNB at two Egyptian ICUs. The high rates of BLR-GNB carriage and acquisition in both ICUs call for strict infection

control practices and judicious prescription and use of antibiotics. Further study is needed to evaluate the effectiveness of introducing admission screening for ESBL, AmpC β-lactamase and carbapenemases in Alexandria University Hospitals. Cost-benefit analyses need to be conducted, taking into consideration the potential savings from decreased transmission, both from a financial and public health perspective.

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