

Original

Detection of Extended-Spectrum Beta-Lactamase and Carbapenemase Producing Gram Negative Bacteria Isolated from Intensive Care Unit -Khartoum-Sudan 2022

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ABSTRACT

Background: Carbapenemase are enzymes that are a major cause of bacterial resistance to the Carbapenem family of antibiotics such as meropenem. ESBL are derived from genes for narrow-spectrum beta-lactamase by mutations that alter the amino acid configuration around the enzyme active sites.

Objective: The study aimed to detect the production of carbapenemases and Extended-spectrum beta-lactamase among Gram-negative bacteria isolated from Intensive Care Units (Alselah Hospital, Ribat University Hospital, and Sharg Elnile Hospital in Khartoum state, Sudan.).

Methodology: Descriptive cross-sectional facility-based study, conducted in Alselah Hospital, Ribat University Hospital, and Sharg Elnile Hospital in Khartoum state, Sudan, in which 100 gram negative bacteria were isolated from ICU (by simple random technique) in the period June to October 2022, and were investigated for susceptibility, phenotypic detection of carbapenemases and ESBL. The isolated bacteria were purified by subculture in MacConkey agar, colonial morphology, gram stain and set of biochemical tests were done and antimicrobial susceptibility test was performed by Carbapenem antibiotic family. Finally the phenotypic detection of carbapenemases was done by modified Hodge

test, and then used double disk diffusion method to detect extended spectrum beta lactamase.

Result: One hundred isolates were investigated in this study, when susceptibility test was performed the highest susceptibility rate was registered for meropenem (84%) and the highest resistance rate for ceftriaxone (55%). ESBL producing bacteria were (16%) and Modified Hodge Test positive was (3%).

Conclusion: The findings revealed that there was a minimum rate of ESBLs and Carbapenemase producing gram negatives isolated from ICU.

Key words: gram negative, carbapenemases, ESBL, ICU, MHT.

Introduction

Gram negative bacteria (GNB) have simple dietary needs and are straight rod-shaped, non-sporulated, non-motile, or motile thanks to flagella, facultative anaerobes, oxidase-negative or positive, catalase-positive, and nitrate to nitrite converters. Due to their high level of antibiotic resistance, gram-negative bacteria are one of the biggest global health issues. Because of the substantial risk they pose to patients in the intensive care unit (ICU) and the high morbidity and mortality they cause, these organisms have clinically meaningful significance in facilities [1]. Third-generation cephalosporins alterations in the enzymes' substrate spectra might lead to β -lactamase-mediated resistance to β -lactam drugs and aztreonam can both be hydrolyzed by extended-spectrum β -lactamases (ESBLs), a rapidly developing class of β -lactamases that is blocked by clavulanic acid. They show for the first time how basic [2]. In 1983, the first description of plasmid-encoded β -lactamases that could hydrolyze extended-spectrum cephalosporins appeared [3]. Most commonly found in *Klebsiella spp* and *E. coli*, as well as other Enterobacteriaceae families like *Enterobacter spp.*, *Proteus spp.*, *Citrobacter spp.*, *Morganella spp.*, *Providencia spp.*, *Salmonella spp.*, and

Serratia spp., ESBL-producing Enterobacteriaceae are important members of antibiotic-resistant bacteria that cause hospital and community acquired infections [4]. Antibiotics named carbapenems are now only used as the last option to treat infections caused on by multidrug-resistant Gram-negative bacteria, according to reports, the mortality rate among MBL producer's ranges from 18 to 67, the carbapenemase enzyme breaks down a class of antibiotics known as carbapenems [5]. Carbapenemase are difficult to detect, both phenotypic and genotypic techniques can be used to detect it, MHT is one of the most straightforward phenotypic tests that may be carried out in a lab [6].

A study done in Soba University Hospital in Sudan revealed that 60% of inpatients receive antibiotics on their first day of admission. Resistance of antimicrobial resistance is greatly influenced by antibiotic misuse; Resistance to antibiotic drug therapy is an increasing public health problem in all populations. In the recent years, through the abuse and misuse of antibiotics, many bacteria have developed resistance to the variety of antibiotics. ESBLs or β -lactamases has probably resulted in the selection, survival and propagation of these organisms. This could have contributed to carbapenem resistance,

this adds to the problem of antimicrobial resistance that continues to threaten public health. Carbapenem-resistant gram-negative bacteria (CR-GNB) are an evolving cause of both community-acquired and healthcare-associated infection (HCAI) that pose a significant threat to public health. The present prevalence rate of 3.8% carbapenem resistance is lower than a recent report from Khartoum of 23.2% [8]. Gram negative bacteria [7]. Study done in Soba University Hospital in Sudan revealed total of 1129 Gram negative bacilli were isolated Forty-three (3.8 %) of them were resistant to carbapenem. The most common of these isolates was *Acinetobacter baumannii*, accounting for 14 isolates, followed by ten *Klebsiella pneumoniae* and seven *Pseudomonas aeruginosa*. Twelve other Gram-negative bacteria. Carbapenem resistance is not well established in Sudan. Resistant gram-negative bacteria are an emergent source of both community-acquired and healthcare-associated infection that poses a substantial hazard to public health [8].

Materials and Method

This study was approved by the ethical committee of the program of Medical Laboratory Sciences at Napata College.

Permission from hospital was applied and verbal consent was taken from participants involved in the study.

A descriptive cross-sectional facility-based investigation of 100 gram negative bacteria isolated from ICU patients in Khartoum state, the recovered bacteria were purified by cultivating in the MacConkey agar. In addition to performing colonial morphology, gram stain, a variety of biochemical assays, and an antimicrobial susceptibility test double disc diffusion method was performed to detect presence of ESBL production the antibiotic family carbapenem, The test is performed on agar with a 30-lg disk of cefotaxime (and / or ceftriaxone and / or ceftazidime and / or aztreonam) and a disk of amoxicillin-clavulanate (containing 10 lg of clavulanate) positioned at a distance of 30 mm (center to center), i.e., at the distance provided by several types of disk-dispenser. Positive test showed a characteristic shape zone referred to as keyhole when a decreased susceptibility to cefotaxime is combined with a clear-cut enhancement of the inhibition zone of cefotaxime in front of the clavulanate-containing disk. [9] The modified Hodge test was utilized to phenotypically identify carbapenemase production. The test was performed by a 0.5 McFarland dilution of the *E. coli* ATCC 25922 in 5 ml of broth

or saline was prepared A 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate. A 10 ug meropenem or ertapenem susceptibility disk was placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35 °C

in ambient air for 16-24 hours. Positive test showed a cloverleaf-like indentation of the E. coli 25922 growing along the test organism growth streak within the disk diffusion zone.[6]

Data was analyzed using the statistical package for social science (SPSS) and Microsoft excel.

Results

Table 1: Demographic variables of ICU patients and frequency of samples of isolated organisms for detection of ESBL and Carbapenemase production.

Variables	Categories	Number	Percent
Age group	Less than 10	3	3%
	21-30	10	10%
	31-40	18	18%
	41-50	12	12%
	51-60	16	16%
	61-70	19	19%
	71-80	16	16%
	81-90	4	4%
	91-100	2	2%
Gender	Male	58	58%
	Female	42	42%
Sample	Swab	64	64%
	Urine	20	20%
	Blood	12	12%
	Sputum	4	4%
Organism	<i>Klebsiella. pneumoniae</i>	41	41%
	<i>Proteus. mirabilis</i>	8	8%
	<i>Proteus. Vulgaris</i>	2	2%
	<i>Escherichia. Coli</i>	19	19%

<i>Citrobacter. Freundii</i>	2	2%
<i>Enterobacter. aerogenes</i>	2	2%
<i>Pseudomonas. aeruginosa</i>	19	19%
<i>Acinetobacter. baumanii</i>	7	7%

The most frequent age group in this study was (61-70) and 58% of participant in this study was male. The most isolated bacteria were *K. pneumoniae* (41%) and the majority of samples taken from patients were swab (64%) in gram negative bacteria isolated from intensive care unit

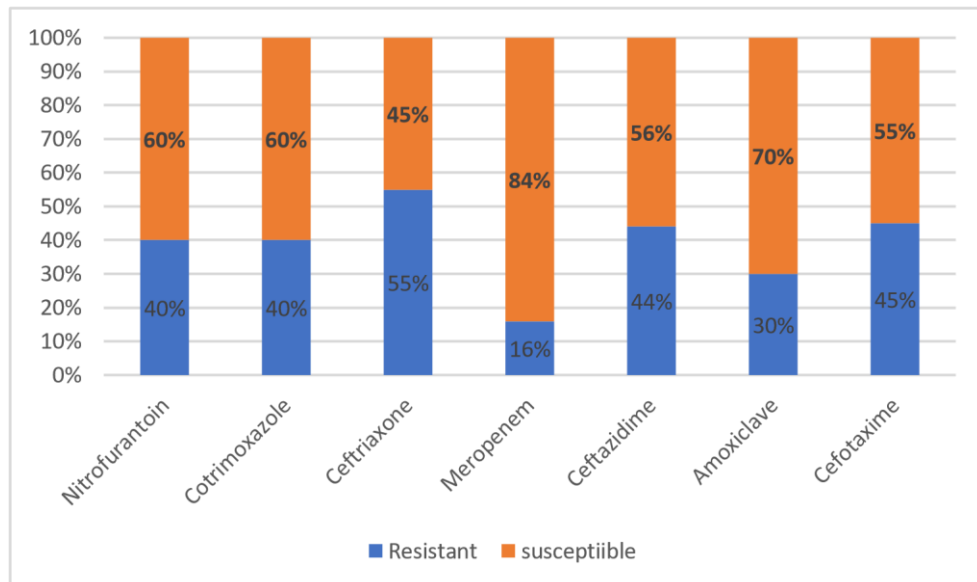


Figure 1: Distribution of Antimicrobial susceptibility test results

The highest susceptible antibiotic is meropenem (84%) and the highest resistance is ceftriaxone (55%) in gram negative bacteria isolated from intensive care unit.

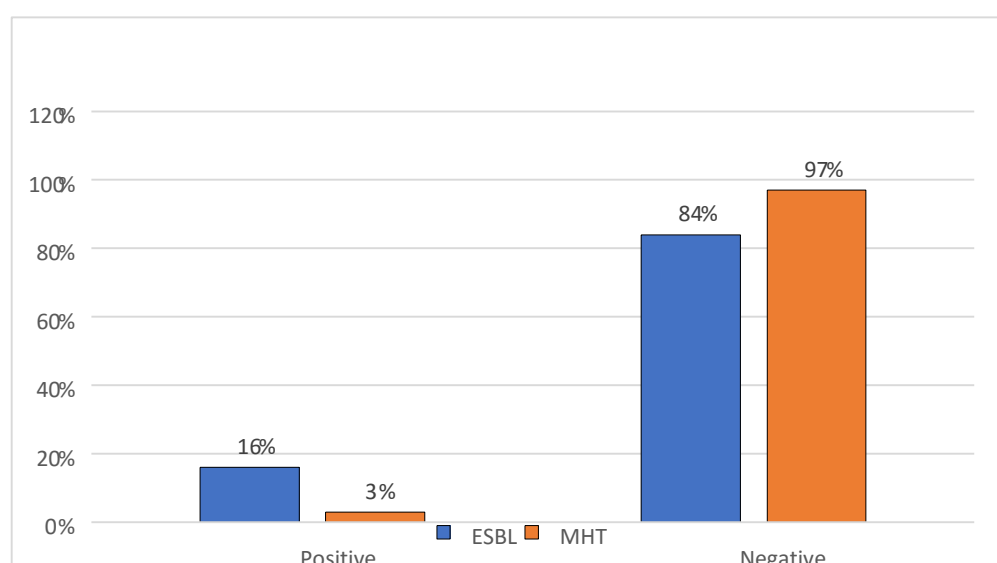


Figure 2: Frequency of ESBL and MHT positive

The frequency of ESBL in this study is (16%) And frequency of MHT is (3%) in gram negative bacteria isolated from intensive care unit.

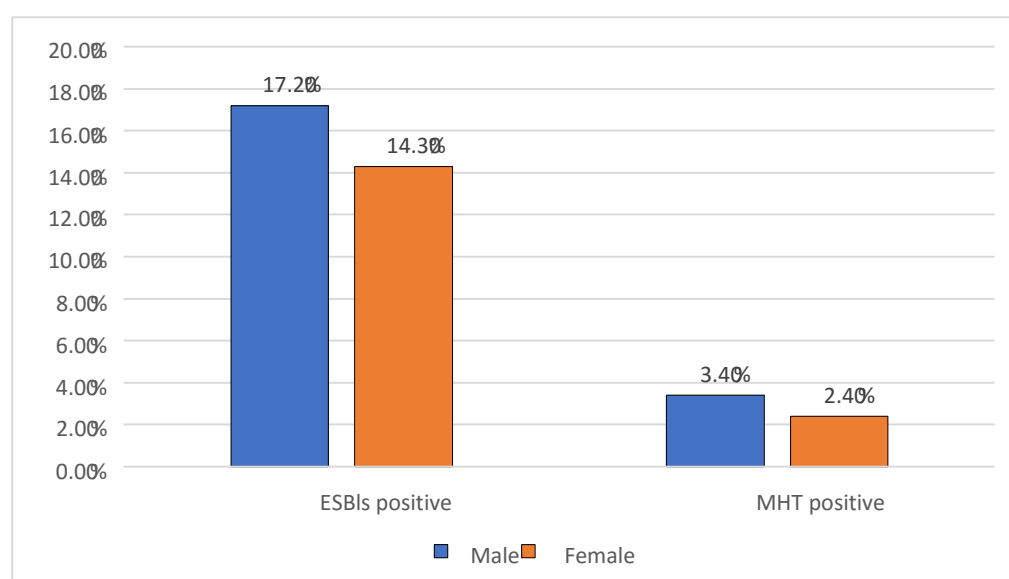


Figure 3: Distribution of ESBL and MHT positive results regarding the gender

The highest ESBL positive distribution rate in male (17.20%) and MHT positive was also in male (3.40%) in gram negative bacteria isolated from intensive care unit.

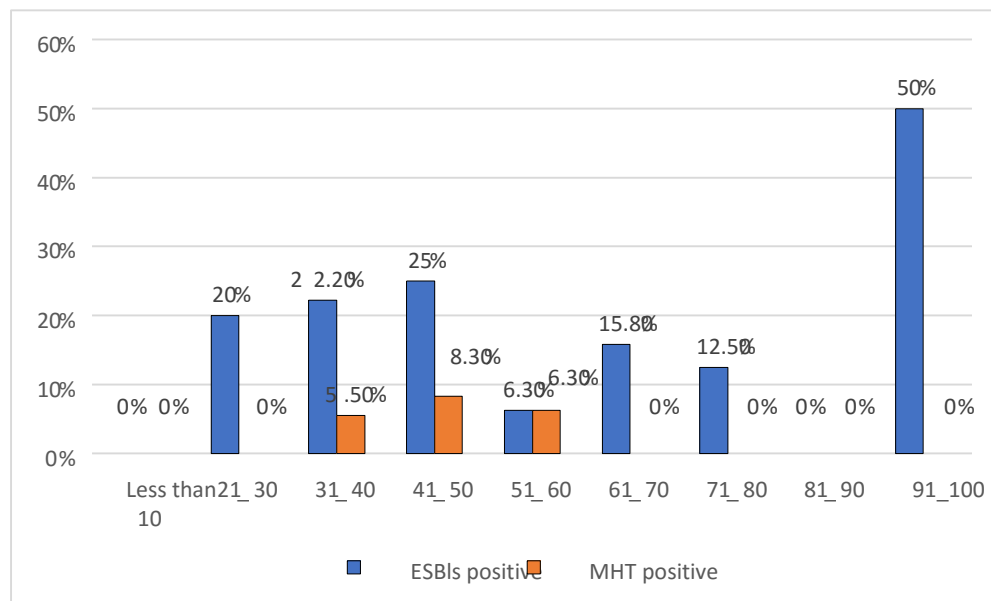


Figure 4: Distribution of ESBL and MHT positive results regarding the age group

The majority of the age groups that were positive in ESBL are between (91-100) (50%) And in MHT were between (41-50) (8.30%) in gram negative bacteria isolated from intensive care unit.

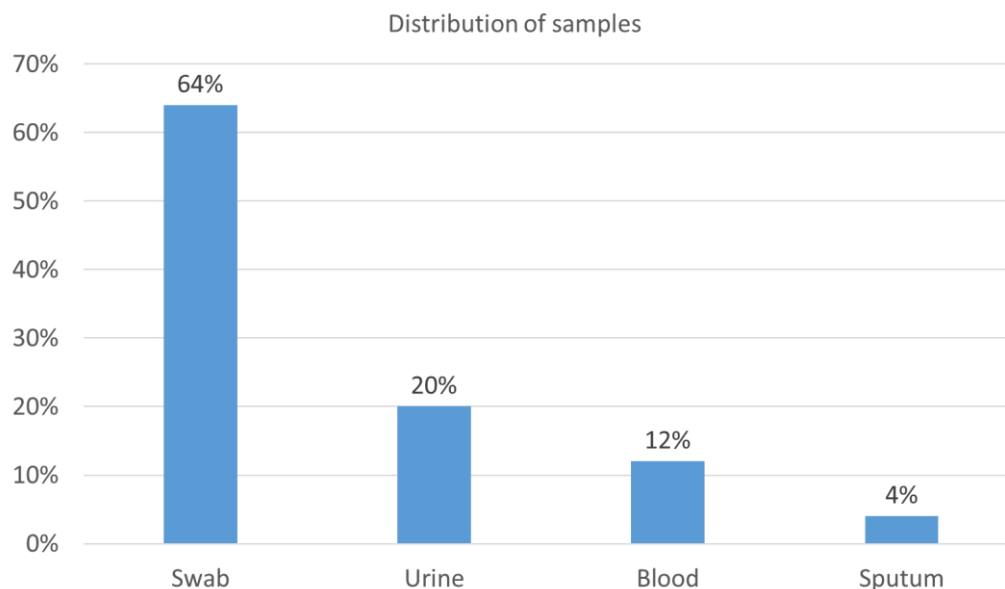


Figure 5: Distribution of ESBL and MHT positive results regarding the type of sample

The majority of samples taken from patients was swab (64%) in gram negative bacteria isolated from intensive care unit.

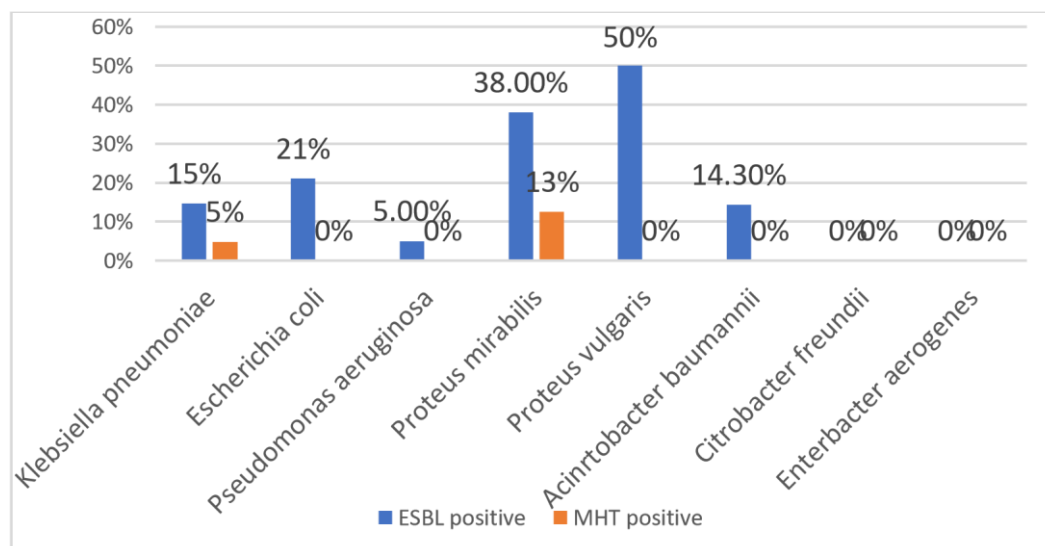


Fig 6: Distribution of ESBL and MHT positive results regarding the isolated organism

The most isolated bacteria are *P.vulgaris* (50%) in gram negative bacteria isolated from intensive care unit.

Discussion

Resistance to antibiotic drug therapy is an increasing public health problem in all populations. Bacteria can rapidly develop resistance to new antimicrobial agents a few years after they become available for commercial use. This study considered and measured the resistance of several Gram-negative bacteria to meropenem and other selected antibiotics which showed that higher susceptible rate was registered for meropenem (84%)

and Amoxiclavate (70%) respectively while the highest resistance rate was registered for ceftriaxone (55%), in a study conducted by Othman et al in Khartoum, Sudan 2019, which showed that higher resistance rate registered to imipenem was (17.2%) so they disagree with our study because Othman et al worked in *E. Coli*, *K.pneumoniae*, *P. aeruginosa* while we worked with various GNB [10]. In the current study we found that the meropenem resistance was 16% while in another study by Gupta et al in

north India 2006 ,showed the prevalence of carbapenem resistance in different GNB ranging from 17% for meropenem and 22% to imipenem , this study agreed to our's due to similarity in that they worked on isolates from ICU and non ICU and found that the higher resistance rate was in ICU isolates [11] .In a study carried out in Spain in 1998 by Pena C et al it was observed that 45% and 86.0% of cefotaxime and ceftazidime resistant *K. pneumoniae* strains, respectively, were also ESBL producers while in the present study the ESBL production was 16% [12]. The disagreement maybe due to that Pena C et al choose only *K. pneumoniae* while we worked in different GNB. The most frequent ESBL production in the age group is between (91-100) (50%) and we compare that with a study which was conducted by Alebel et al in Ethiopia 2021 [13] that overall 67 (24.8%) patients had infection with ESBL most of them are Male producing GNB highest percentage was recorded among isolates from wound swab (81.3%) but the majority of our isolates was from sputum, the disagreement is because of the difference in overall sample count and type of sample .We compare the

results of MHT with another study that was in Eastern India by Datta et al in 2017 [14], Out of the 132 isolates screened resistant during the study period, 75% (99/132) were positive for MHT , The MHT positive results in male age grouped from (41-50) was (3.40%) 3/100 ,The difference in results and percentages is a result of the different geographical areas and size of samples *E .coli*, *K. pneumoniae*, *P. aeruginosa* ,*P. mirabilis* and *A. baumannii* all had extended spectrum B- lactamase at rates of 19 (21%) ,41 (15%),19 (21%) , 8 (38%) and 7(14.3%) our percentages are lower than the finding of Jain et al [15] 2003 who found that 14%),(24.6%),(2.9%)and (1.7%) For *E. coli* , *klebsiella spp* ,*pseudomonas spp* , *Acinetobacter spp* concurrently. The epidemiological aspect may be affecting the results because of how we deviated from the previous study Percentages of Modified Hodge test which was distributed on two clinical isolates as following: (5%) in *K. pneumoniae* and *P. mirabilis* (13%) which in Lee et al Korea 2009 [16] Reported higher rate among *K. pneumoniae* While Pal et al in India 2016 [17] study have shown lower prevalence of *proteus* isolated (101 /1876) patients , Among the 101

isolates included in the study, 92 isolates were found to be screened positive for ESBL Resistance to carbapenems in the present study was (12.5%) . We disagree with Lee et al regarding the type of organism that was determined in their investigation. The current study's findings concur with those of Pal et al, who claimed that proteus spp made up 13% of ESBLs. In present study highest ESBL in male (17.3%) while in female it was (14.3%) and the MHT positive was also highest in male more than female (3.4%),(2.4%) respectively, and this is agreed with another study conducted in Egypt by Nageeb et al 2014 [18] which revealed that the MHT was found in male more than female (52.2%), (47.7%) respectively and this similarity referred to the same geographical area in which two studies were conducted .

Conclusions and Recommendation

- The frequency of carbapenemase producing bacteria was found to be (3%).in addition to considerable percentages of ESBLs producing bacteria that were reported by (16%).
- The prescription of antibiotics should base on susceptibility testing.
- Complete dose of antibiotic must be taken to prevent resistance.
- Policies should be taken by health authorities to minimize the risk of antibiotic resistance.
- Techniques for further identification of resistance gene regarding ESBLs and carbapenemase may be required.

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Ethical approval

This study was approved by the ethical committee of the program of Medical Laboratory Sciences at Napata College. Permission from hospital was applied and verbal consent was taken from participants involved in the study.

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baumannii isolates from intensive care units at a tertiary-care hospital in

Egypt. EMHJ- Eastern Mediterranean Health Journal 2014,20:3, 203-211.

List of abbreviations

ICU	Intensive Care Unit
ESBL	Extended Spectrum Beta-lactamase
MHT	Modified Hodge Test
GNB	Gram Negative Bacteria
CR-GNB	Carpabenemase Resistant Gram Negative Bacteria