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ORIGINAL ARTICLE

Characterization of Non- Fermenter Gram- Negative Bacilli infection in Intensive Care Unit of National Liver Institute

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ABSTRACT

Background: *Bacilli infections is a leading cause of mortality and morbidity for patients with cirrhosis especially with ICU hospitalization and overuse of antibiotics all complicate patient's prognosis. NFGNB are heterogenous group of gram -negative bacilli that emerged as hospital acquired pathogens.* ***Objectives:*** *To characterize non - fermenter gram negative bacilli isolated from different clinical specimen of the ICU admitted hepatic patients and assess if hospital environment and workers share in its spread.* ***Methodology:*** *This is a cross sectional observational study done on liver disease patients admitted to The National Liver Institute, Menoufia University, included ICU environmental samples and HCWs samples, where samples for bacteriological cultures were collected and cultivated by ordinary methods and Organisms were identified by biochemical and confirmed by VITEK 2 system. Antibiotic Resistance pattern was confirmed by automated system, E-test. Results were tabulated and analyzed.* ***Results:*** *NFGNB infection was prevalent among ICU hospitalized end stage liver disease patients representing (27.4%), environmental samples group which represent (12.5.5%) and HCWs group which represent (7.5%). Of which pseudomonas aeruginosa, Acinetobacter baumannii in patient samples were significantly higher than other groups. Higher percentage of MDR-NFGN and XDR-NFGN were predominant among isolates from all groups, but all are Colistin sensitive. Higher incidence of ESBLs producer NFGNB include Achromobacter xylosoxidans and Aeromonas hydrophila isolate as they represent (100%) followed by Burkholderia cepacia which represent (66.7%) while the highest incidence of MBL positive test were among Achromobacter xylosoxidans and Aeromonas hydrophila isolates followed by pseudomonas. E-test remains the golden test in detection of ESBL and MBL-NFGN.* ***Conclusion:*** *Resistant NFGNB is an emerging bacterium that threats hepatic patients 'outcome, especially pseudomonas aeruginosa, Acinetobacter baumannii at the front position for more studies regarding different patterns of antibiotic resistance in ICU.*

INTRODUCTION

Bacterial infections in patients with end-stage liver disease increases mortality to more than 50% and is associated with significant costs. Changes in gut bacilli in cirrhosis can lead to bacterial overgrowth with subsequent enhanced bacterial translocation from the gut to the systemic circulation and ascites, identified by bacterial DNA or by isolating bacilli in systemic biofluids[1].

The non-fermenter, is a heterogenous group of gram-negative bacilli (NFGN) that is aerobic, can't ferment sugars, but use them through the oxidative route which triggers severe, fatal hospital infections, especially in ICU patients who undergo invasive procedure including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia*, *Stenotrophomonas*., *Alcaligenes*, *Moraxella* [2].

Intrinsic resistance is the main problem of **NFGNB** since they produce genes with multiple mechanisms capable of modifying the microbicidal action [3]. *P. aeruginosa*, produce cephalosporinase of type AmpC, and efflux systems that confer resistance to b-lactams as MexAB-OprM; and loss of OprD (which gives impermeability to the bacterial cell due to the loss of porin)[4]. *Acinetobacter baumannii* naturally produces AmpC cephalosporinase and oxacillinase (OXA), leaving it spontaneously immune to many drugs in addition to high impermeability combining with the resistance of mechanisms such as extended-spectrum b-lactamases (ESBL) [5]. The *Stenotrophomonas* exhibit a pattern of intrinsic multi-resistance, especially in patients who have had contact with carbapenems.

Thus, *Stenotrophomonas* present several efflux pumps and produce two carbapenemases – L1 (resistance to all carbapenems) and L2 (cephalosporinase), in synthesis [6]. These mechanisms, associated or separate, restrict the treatment options to an alarming level. Although it is sensitive to sulfamethoxazole-trimethoprim and has an increased resistance mediated by plasmids [7]. The problem of multi-resistant NFGN infections is on the rise since the beginning of the 21st century. However, there is difficulty eradicating drug-resistant organisms because of the lack of effective antibiotics[2]. The aim of this study was to characterize infections in patients with end-stage liver disease caused by NFGN and to detect the role of hospital workers and environment in the rate of infection.

METHODOLOGY

The present cross-sectional study was carried out during the period from March 2019 to March 2020 in Intensive Care Units of National Liver Institute, Menoufia University. The study protocol was approved by the local Ethics Committee of the National Liver Institute, Menoufia University. Informed consents were taken from the patients before the beginning of the study following the declaration of **Helsinki**. Groups involved in this study were classified into the followings:

1- Patients group:

It included 358 samples taken from 258 infected patients who were admitted to intensive care units of Liver Institute Hospital. (106 males and 152 females). Clinical samples include blood, urine, sputum, T. tube (TT) and CVP. All the selected patients had infections that became evident 48 hours or more after hospital admission. Single sample was collected per infection site.

Data were collected for each patient including:

Personal and clinical data (Age, sex, cause of hospital admission, date of admission to ICU & date of acquiring nosocomial infection), **Co-morbid conditions** (hepatic disease, diabetes) and prolonged use of antibiotics and History of recent surgery or use of invasive medical devices (as ventilators, catheters).

2- ICU Personnel group:

The second group was samples taken from 40 hospital staff (doctors, nurses, workers) who were in close contact to ICU patients, it included 120 samples were collected from their throats, nose and hands.

3-ICU environment and equipment group

Fifty-six environment and equipment samples were collected from the ICUs included: wall, floor, beds, ventilators, suction device system, dressing baths and antiseptic solutions.

Collection and transport of samples:

All the selected clinical and hospital environmental samples were immediately sent to microbiology lab within two hours to be processed and examined.

Cultivation and isolation of organisms [8]:

Each sample was inoculated on nutrient agar, blood agar, MacConkey agar, mannitol salt agar, and Cetrimide agar plates and incubated aerobically at 37°C for 24-48h. For each urine sample, counting of the microorganisms was done by using sterile calibrated loop (0.001 ml) of well mixed uncentrifuged urine and spread across culture media, after incubation at 37 °C overnight, the number of colonies was multiplied by 1000 to detect the significant bacteruria. - Blood culture: all inoculated bottles were incubated at 35-37°C up to 7 days in BACT/ALERT automated blood culture (Biomeriuex, France) and subcultured when give growth indication on blood agar and Mac Conkey medium

Identification of isolates:

Bacilliculture was subjected to further morphological and biochemical identification to identify different bacillispecies according to the standard microbiological methods**.** [9]

Identification of NFGNB

NFGNB were primary identified based on culture characteristic, morphology, and biochemical tests then, confirmed using VITEK2 compact device system *(Biomeriuex, France)*.

Antibiogram of NFGNB isolates

Antibiotic susceptibility of NFGN isolates was tested using disk diffusion method and confirmed by VITEK2 compact device system **(Biomeriuex, France)** interpreted according to the methods of the Clinical and Laboratory Standards Institute (**CLSI**) formerly National Committee for Clinical Laboratory Standards [10].

Screening for ESβL and MβL using disk diffusion method:

Using disk diffusion method, by noting specific zone diameters which indicate a high level of the multi-resistance of *NFGNB* against ceftriaxone, ceftazidime, cefotaxime and aztreonam indicated suspicion of ESβL production. [11].

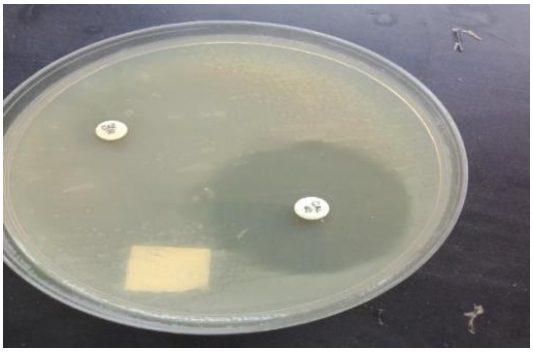

Fig. 1: Phenotypic confirmation tests for ESβL production by double disk diffusion using cefotax30 g and Cefotax plus Clavulinic acid

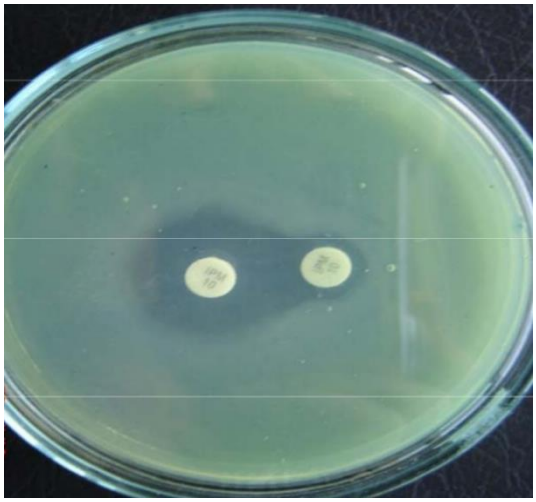

Fig. 2: An increase in the zone around the IMP + EDTA disk than IMP disk alone by > 8 mm (positive for MβL production)

Fig.3: Acinetobacter baumannii MBLs producer confirmed by E- test.

Statistical analysis:

Data were analyzed using IBM SPSS statistics version 21 (SPSS Inc., Chicago, IL). The mean and standard deviation. A p-value < 0.05 was considered significant. MIC and inhibition zone values were calculated.

RESULTS

The present study was conducted during the period from March 2019 to March 2020 in Intensive Care Units of Hepatology Department of The National Liver Institute, Menoufia University. It included 358 samples taken from 258 infected patients after 48 hours from admission (106 males and 152 females) their ages ranged from 20 years to 70 years (mean ± 30.74, in addition to health care workers samples that constitute the second group they were 40 hospital staff (doctors, nurses, workers) who were in close contact to ICU patients, 120 samples were collected from their throats, nose and hands. Also, the study included 56 environment and equipment samples which were collected from the ICUs they included: wall, floor, beds, ventilators, suction device system, dressing baths and antiseptic solutions. mean age of studied patients was significantly higher than that of the HCWs group with (p value=0.004), while sex distribution showed non-significant difference between HCWs group and patients' group. Gram negative bacilli represented the highest frequency ICU organisms in patient group (69.8%) which was significantly higher than other groups (p value<0.001) followed by environmental group which represented about (28.6%) followed by HCWS group which represented about (25%). FNGNB showed the highest distribution in patients' group which was (42.5%) (p value<0.001) followed by environmental group which represented about (16.1%) and HCWS group which represented about (15.8%). patient group showed significant increase in NFGNB which was (27.4%), environmental group which represented about (12.5.5%) and HCWs group which represented about (7.5%). On the other hand, the gram-positive bacilli show significant higher level in HCWS which is (53.3%) then patient group which represented about (19.6%) followed by environmental group which represented about (7.1%). The present study showed that the distribution of NFGN in patients' samples was *Pseudomonas aeruginosa* (12.8%), *Acinetobacter baumannii* (6.1%), *Achromobacter Xylosoxidans* (3.1%), *Stenotrophmonas maltophilia* (2.8%), *Burkholderia cepacia* (1.7%), *Aeromonas hydrophila* (0.8%) and *Burkholderia pseudomallei* (0.6%), which was significantly higher than in HCWs group, NFGN burden was *pseudomonas aeruginosa* (5.0%), *Stenotrophmonas maltophilia* (2.5%), and in ICU environment, pseudomonas aeruginosa represented (12.5%) of total isolates as shown in Figure 4.

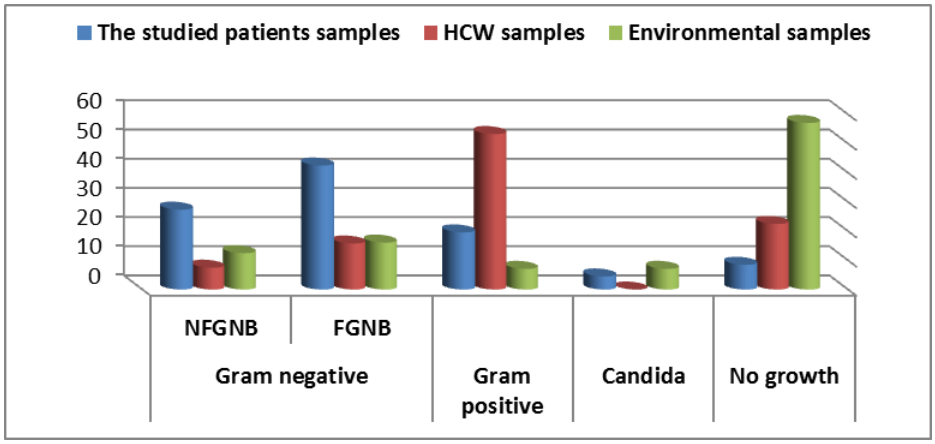

Fig.4: Organisms detected in different studied groups.

In figure 5; NFGNB was significantly increased in sputum than in other patient samples. Urine samples showed significant increase in FGNB. On the other hand, there was no statistically significant difference between NFGNB and FGNB isolated from blood, TT and CVP samples.

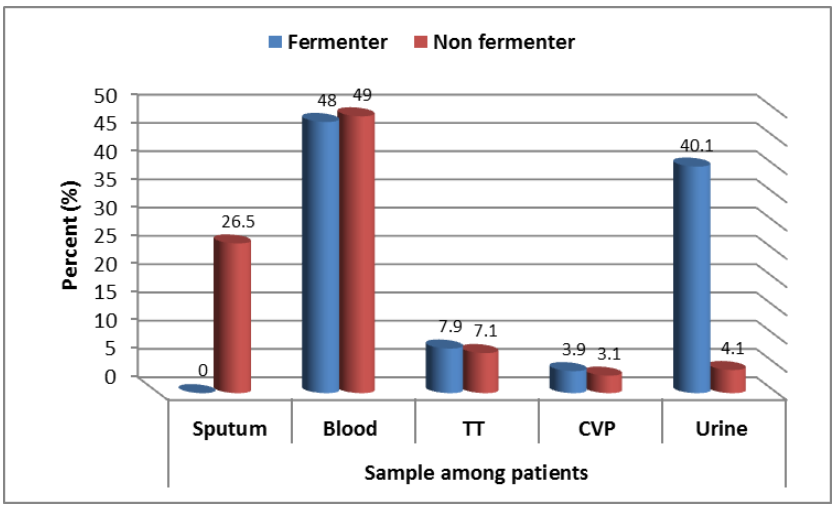

Fig. 5: Distribution of NFGNB in the collected samples.

Table 1: Relation of non-fermenter bacilli in patients' samplesin relation to their socio-demographic and clinical data

Organism	Organisms						
	***Pseudomonas* N = 46**	***Acinetobacter* N = 22**	***Stenotrophomonas maltophilia* N = 10**	***Burkholderia cepacia* N = 6**	***Achromobacter Xylosoxidans* N = 11**	***Aeromonas hydrophila* N = 3**	***Burkholderia pseudomallei* N = 2**
Age (years)							
Mean±SD	48.6±24.8	27.6±21.6	35.6±22.1	31.3±21.5	23.5±10.1	22.7±17.9	40.5±22.02
Range	0.04 – 75	0.05 – 70	3 – 66	8 – 56	10 – 39	2 – 33	2 – 19
Sex [n (%)]							
Male	46 (100%)	19 (86.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Female	0 (0.0%)	3 (13.6%)	10 (100%)	6 (100%)	11 (100%)	3 (100%)	2 (100%)
Drugs							
Corticosteroid	6 (13.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Excessive use ofantibiotics	13 (28.3%)	4 (18.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cholecystectomy	16 (34.8%)	2 (9.15)	0 (0.0%)	0 (0.0%)	4 (36.4%)	0 (0.0%)	0 (0.0%)
Liver transplantation	20 (21.7%)	4 (18.2%)	0 (0.0%)	4 (66.7%)	6 (54.5%)	2 (66.7%)	0 (0.0%)
Type of device Ventilator	14 (100%)	8 (100%)	0 (0.0%)	4 (66.7%)	6 (100%)	2 (66.7%)	0 (0.0%)
Urinary catheter	0 (0.0%)	0 (0.0%)	8 (100%)	2 (33.3%)	0 (0.0%)	1 (33.3%)	2 (100%)

Table 1 showed that the risk factors of Pseudomonas infection were male aged (48.6±24.8) with overuse of antibiotics, corticosteroid, and ventilation while the incidence of *Stenotrophmonas maltophilia* was common in female patients who undergo urinary catheterization. *Burkholderia cepacia*, *Achromobacter Xylosoxidans* and *Aeromonas hydrophila* were associated with female gender, ventilator associated, liver transplantation and prolonged device utilization.

Table 2: Antibiotic resistant pattern of NF Gram negative bacilli isolated from patients' group: -

Organism in clinical isolates	Multidrug resistance MDR (%)	Extreme drug Resistance XDR (%)	Test	P value
Acinetobacter (*n = 22)*	10 (45.5.0%)	12 (55.5%)	1.92	0.06
Pseudomonas *(n = 46)*	32 (47.8%)	24 (52.2%)	2.41	**0.01***
Stenotrophomonas maltophilia *(n = 10)*	3 (30.0%)	7 (70.0%)	0.94	0.35
Burkholderia cepacia *(n = 6)*	4 (66.7%)	2(33.3%)	1.17	0.24
Aeromonas hydrophila *(n = 3)*	2 (66.7%)	1 (33.3%)	0.87	0.39
Burkholderia pseudomallei *(n = 2)*	1(50%)	1(50%)	1.0	0.32
Achromobacter Xylosoxidans *(n = 11)*	7 (63.6%)	4 (27.3%)	2.21	**0.02***

- **MDR** was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories
- **XDR** was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacilli isolates remain susceptible to only one or two categories)

Table 2 showed that in clinical isolates there were: ((45.5.0%) of *Acinetobacter*, (47.8%) of *pseudomonas spp*, (30.0%) of *Stenotrophomonas maltophilia*, (66.7%) of *Burkholderia cepacia*, (66.7%) of ***Aeromonas hydrophila***, (50%) of ***Burkholderia pseudomallei*** and (63.6%) of ***Achromobacter Xylosoxidans*** were MDR while (55.5%) of Acinetobacter, (52.2%) of pseudomonas spp, (70.0%) of ***Stenotrophomonas maltophilia***, (33.3%) of ***Burkholderia cepacia***, 2 (33.3%) of ***Aeromonas hydrophila***, (50%) of ***Burkholderia pseudomalle***i and (27.4%) of ***Achromobacter Xylosoxidans*** were XDR. the incidence of MDR was significantly high in ***Achromobacter Xylosoxidans*** **and** ***pseudomonas aurignosa.***

Table 3: Antibiotics resistance pattern of NF Gram negative bacilli isolated from HCWs group:-

Organism in HCWs isolates	Multidrug resistance MDR (%)	Extreme drug resistance XDR (%)	Test	P value
Pseudomonas *(n = 6)*	5 (83.3%)	1(16.7%)	0.61	0.54
Stenotrophomonas maltophilia *(n = 3)*	2(66.6 %)	1(33.3%)	0.0	1.0

Table 3 showed that (83.3%) of Pseudomonas, (66.6%) of ***Stenotrophomonas maltophilia*** were MDR while (16.7%) of Pseudomonas, (33.3%) of ***Stenotrophomonas maltophilia*** were XDR which was statically insignificant.

Table 4: Antibiotics resistance pattern to antibiotics of pseudomonas isolated from environmental samples: -

Organism in Environmental isolates	Multidrug resistance MDR (%)	Extreme drug Resistance XDR (%)	Test	P value
Pseudomonas *(n = 7)*	4 (57.14)	3 (42.9%)	0.0	0.99

Table 4 showed that (57.14 %) of *Pseudomonas* were MDR while (42.9%) were XDR which was statically insignificant.

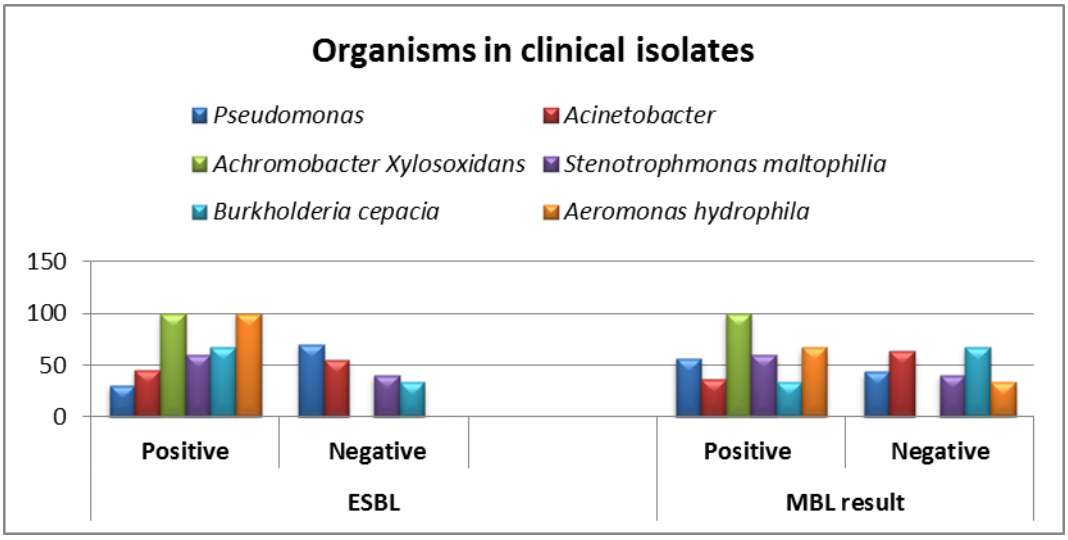

Fig.6: showed that the highest incidence of ESBLS positive test were among *Achromobacter Xylosoxidans* and *Aeromonas hydrophila* infections as they represent (100%) followed by *Burkholderia cepacia* which represent (66.7%) while the highest incidence of MBL positive test were among *Achromobacter Xylosoxidans* and *Aeromonas hydrophila* patient infection followed by *pseudomonas*.

Table 5: Beta lactamase producer *Pseudomonas aeruginosa* in different studied sample groups: -

	***Pseudomonas aeruginosa* in different samples**			**Test**	**P value**
	Patients N = 46 (%)	**HCWs N = 6 (%)**	**Environmental samples N = 7 (%)**		
ESBL				2.5	0.11[1]
Positive	32(69.6%)	6 (100%)	7 (100%)	12.3	<0.001[2**]
Negative	14(30.4%)	0 (0.0%)	0 (0.0%)	13.0	<0.001[3**]
MBL result				0.09	0.76[1]
Positive	26(56.5%)	3(50.0%)	7 (100%)	4.89	0.03[2*]
Negative	20(43.5%)	3(50.0%)	0 (0.0%)	4.55	0.03[3*]

X^2 = Chi square test
1: comparing patients' samples with HCWs samples
2: comparing patients' samples with environmental samples
3: comparing HCWs samples with environmental samples
*p value<0.05(significant)
**p value<0.005(highly significant)

Table 5 showed comparing ESBL test of ***Pseudomonas aurignosa*** isolates in patients' samples with environmental samples which is statically highly significant as (p value <0.001) and comparing HCWs samples with environmental samples which were statically highly significant as (p value <0.001).

Table 6: No & % of MβL producing *NFGNB* detected by phenotypic tests in patient group.

Micro Organism	No & % of positive organisms with		
	Disk diffusion Method (%)	DDT (%)	E-test (%)
Pseudomonas* *N =46	26(56.5%)	26(56.5%)	22(47.8%)
Stenotrophomonas maltophilia* *N = 10	6(60%)	6(60%)	4(40%)
Acinetobacter* *N = 22	14(63.6%)	14(63.6%)	16(72.2%)
Achromobacter Xylosoxidans N = 11	3(27.2%)	3(27.2%)	2(18.1%)
Burkholderia cepacia* *N = 6	2(33.3%)	2(33.3%)	1(16.6%)
Aeromonashydrophila* *N = 3	2(66.6%)	2(66.6%)	1(33.3%)
Burkholderia pseudomali=2	1(50%)	1(50%)	1(50%)
Total=100	53(53%)	53(53%)	47(47%)

Table 6 showed that MβL was produced by (56.5%) of Pseudomonas isolates, (63.6%) of Acinetobacter isolates, (66.6%) of *Aeromonas hydrophila* isolates, 60% of *Stenotrophmonas maltophilia* isolates and 27.2% of *Achromobacter Xylosoxidans* isolates detected by disk diffusion, while E test detected the presence of MβL in (47.8%) of Pseudomonas isolates, (72.2%) of Acinetobacter isolates , (33.3%) of *Aeromonas hydrophila* isolates, 40% of *Stenotrophmonas maltophilia* isolates and (18.1%) of *Achromobacter Xylosoxidans* Isolates.

Table 7: Comparison of double disk synergy test (DDT) and E-test for detection of MβL among *Pseudomonas spp* and *Stenotrophmonas maltophilia* in health care workers samples

MBL		DDT	E-test
Pseudomonas **N =6**	**MβL +ve**	**3**	**2**
	MβL –ve	**3**	**4**
	X^2 (P-value)	0.34 (0.56)	
Stenotrophmonas maltophilia **N = 3**	**MβL +ve**	**0**	**1**
	MβL –ve	**3**	**2**
	X^2 (P-value)	1.2 (0.27)	

The table 7 showed the comparison between the double disk synergy test and E test for detection of MβL and showed a non- significant difference between the results of both tests.

DISCUSSION

NFGN bacilli including *Pseudomonas aeruginosa, Acinetobacter baumanii, Borkhorderia cepacia* and *Stenotrophomonas maltophilia* are adaptable pathogens with a high level of variable virulence that are responsible for increasing infection in liver disease patients. It is one of the most important nosocomial pathogens can cause severe infections such as bacteremia, urinary tract and device associated infections in hospitalized and immunocompromised patients[12].

Bacilli infections are considered a leading cause of mortality and acute-on-chronic liver failure in end-stage liver disease (ESLD) that causes prolonged hospital to stay, acute kidney injury (AKI), death, decrease chance for liver transplantation and more susceptibility to further infections[13]. The aim of this study was to describe NFGN bacilli infections in end stage liver disease patients admitted to ICU and their pattern of antibiotic resistance and if the ICU environment favors its presence.

In the current study, 358 post hospitalization samples were collected from 258 admitted hepatic patients 48 hours after admission, 120 health care workers samples were collected, In addition to 58 samples from the ICU environment. NFGN bacilli isolates were (27.4%) in patient groups, (7.5%) in HCWs group, (12.5%) in Environmental isolates group. Comparable results was obtained by Somily, et al[14] and Balkhy, et al[15].

The present study showed that the distribution of NFGN in patients' samples was *Pseudomonas aeruginosa* (12.8%), *Acinetobacter baumannii* (6.1%), *Achromobacter Xylosoxidans* (3.1%), *Stenotrophmonas*

maltophilia (2.8%), *Burkholderia cepacia* (1.7%), *Aeromonas hydrophila* (0.8%) and *Burkholderia pseudomallei* (0.6%), which was significantly higher than in HCWs group. NFGN burden in HCWs was *pseudomonas aeruginosa* (5.0%), *Stenotrophmonas maltophilia* (2.5%), and in ICU environment, *pseudomonas aeruginosa* represent (12.5%) of total isolates. This result was in agreement with studies with the very close percentages [16] [17] and[18] . On the hand, some studies reported equal burden of *P. aeruginosa* and *A. baumannii* [19]. This may be related to reporting of hospital outbreaks of Acinetobacter during the study time[20].

The current study entailed that the risk factors for Pseudomonas and Acinetobacter infection were male aged (48.6±24.8) years old with overuse of antibiotics, corticosteroid, and mechanical ventilation while the incidence of *Stenotrophmonas maltophilia* was common in female patients who undergo urinary catheterization. *Burkholderia cepacia*, *Achromobacter Xylosoxidans* and *Aeromonas hydrophila* were associated with female gender, ventilator associated, liver transplantation and prolonged device utilization. This findings were in agreement with Abdallah, etal [21] and Rattanaumpawan, etal [22].

In our study, MDR- NFGN isolated from ICU patients were: (66.7%) of *Burkholderia cepacia*, (66.7%) of *Aeromonas hydrophila*, (63.6%) of *Achromobacter Xylosoxidans*, (50%) of *Burkholderia pseudomallei* and (47.8%) of pseudomonas spp, (45.5.0%) of Acinetobacter, ((30.0%) of *Stenotrophomonas maltophilia*, Comparable results was obtained by Siwakoti, S.,et al, 2018 [23] who mentioned that *Acinetobacter* species (41%, 52/128) was the commonest MDR organism followed *Pseudomonas* spp (21%, 27/128) [23]. [24] and [25] reported high percentage of MDR-NFGN isolates.

The current study revealed that (55.5%) of Acinetobacter, (52.2%) of pseudomonas spp, (70.0%) of *Stenotrophomonas maltophilia*, (33.3%) of *Burkholderia* cepacia, 2 (33.3%) of *Aeromonas hydrophila*, (50%) of *Burkholderia pseudomallei* and (27.4%) of *Achromobacter Xylosoxidans* were XDR. These results agreed with Siwakoti, etal [23] and Teerawattanapong, etal [24].

This study showed that showed that in environmental samples (83.3%) of *Pseudomonas*, (66.6%) of *Stenotrophomonas maltophilia* were MDR while (16.7%) of *Pseudomonas*, (33.3%) of *Stenotrophomonas maltophilia* were XDR which was statically insignificant while (57.14 %) of *Pseudomonas* were MDR while (42.9%) were XDR which was statically insignificant. This give evidence that the ICU environment is the main accused source for NFGN infection to ICU hospitalized patients while the HCWs resistance pattern was different from environmental pathogens and those isolated from patients, also give appoint to overuse of hand sanitizers by HCWs elevated the percentage of MDR-NFGN bacilli carriage rate. These results were in accordance with Jean,S, etal[25].

In the current study, highest incidence of ESBLS positive test were among *Achromobacter Xylosoxidan*s and *Aeromonas hydrophila* infections as they represent (100%) followed by Burkholderia cepacia which represent (66.7%) while the highest incidence of MBL positive test were among *Achromobacter Xylosoxidans* and *Aeromonas hydrophila* patient infection followed by pseudomonas. This agreed with Somily, etal, who reported that We are reporting high and/or increasing resistance of NFGNB to common treatment options. The current findings call for urgent actions to combat the increasing resistance of NFGNB.

This study showed that MβL was produced by (56.5%) of *Pseudomonas* isolates, (63.6%) of *Acinetobacter* isolates, (66.6%) of *Aeromonas hydrophila* isolates, 60% of *Stenotrophmonas maltophilia* isolates and 27.2% of *Achromobacter Xylosoxidans* isolates detected by disk diffusion and DDT, while E test detected the presence of MβL in (39.1%) of *Pseudomonas* isolates, (40%) of *Acinetobacter* isolates =, (33.3%) of Aeromonas hydrophila isolates, 40% of *Stenotrophmonas maltophilia* isolates and (18.1%) of *Achromobacter Xylosoxidans* Isolates. the comparison between the double disk synergy test and E test for detection of MβL and showed a non- significant difference between the results of both tests. Comparable results obtained by Abott, etal[26].

In conclusion, NFGN bacilli are a heterogenous group of highly resistant bacilli responsible for bad outcome of hepatic patients that can be transmitted via surrounding environment or HCWs. So, with standard precautions as proper isolation of MDR infected patients, routine hand hygiene, proper sterilization of medical equipment and restrict follow the antibiotic steward ship specific to hepatic patients are the key in holding spread of XDR and MDR bacilli.

Conflict of interest

All authors declare no conflict of interest.

Fund: no fund was received.

Ethical Statement:

Informed consent was obtained from all individual participants included in the study before obtaining their culture sample. All procedures performed in studies involving human participants were in accordance with the ethical standards of the National Liver Institute Research Ethical Committee and with the 1964 Helsinki declaration and its later amendments.

All authors made meaningful contributions to the work. Each contributed to preparation of the manuscript

and agreed to the submission of the final version. There are no conflicts of interest to declare.
Thank you for your consideration.
On behalf of all co-authors.

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