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Correlation Between Gram Negative Bacterial Colonization, Environmental Contamination and the Development of Infections at Intensive Care Unit

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Abstract. Detection of the source of infection by multidrug resistant gram-negative bacteria (MDR-GNB) may help in the development of effective treatment protocols resulting in improved patient outcomes. The aim of this study was to determine the prevalence of MDR-GNB colonization and infection among patients in ICU and the association between colonization, environmental contamination and infections. Throat swabs were taken from 165 ICU patients within 48 h of admission. Then clinical samples were collected from those patients who developed infections according to the site of infection. Also, weekly environmental samples were obtained from the occupied partition in ICU. Throat colonization by GNB was detected in only 6.06%. About 27 (16%) out of the admitted patients got infections and only 9 patients (5.45%) had hospital acquired infections (HAIs). The rate of environmental contamination due to GNB during the studied period (8 weeks) which was 27.06%. MDR-GNB isolates represented 50% of gram-negative isolates recovered from colonized patients and 86.11% from infected patients and 58.08% from environmental samples. *Klebsiella pneumoniae* (*K. pneumoniae*) was the predominant isolate recovered from both colonized and infected patients (50.00% and 38.89% respectively) as well as from environmental samples (48.29%). Extended spectrum beta lactamases (ESBL) and carbapenem resistance were predominant among *K. pneumoniae* isolates from clinical and environmental samples.

High rate of cross contamination between infected patients and their occupied environment was detected among *K. pneumoniae* and *Acinetobacter baumannii* complex (Acb complex) isolates.

Keywords: colonization, environmental contamination, gram negative bacteria, intensive care units

Introduction

The incidence of HAIs in ICUs of four tertiary care Egyptian University Hospitals reached 36.7%. (Hamam *et al*., 2021). Major risk factors of HAIs in the ICU are its environment, medical procedures applied to treat the patient and above all the patient's general health (Wang *et al*., 2023). MDR or extensively drug-resistant (XDR) GNB impose a very high cost on hospitals causing elevated hospitalization time, increased morbidity and mortality especially in ICU (Wang *et al*., 2019). Contamination by GNB may happen by direct patient shedding of micro-organisms to his immediate environment (Russotto *et al*., 2015). It had been reported that GNB have the ability to survive upto several months on dry inanimate surfaces, with extended persistence under lower-temperature and humid conditions (Wißmann *et al*., 2021). It was generally assumed that transmission from exogenous sources is considered the most frequent route (Khan *et al*., 2017). However, this opinion has been remarkably challenged, as a result of awareness being paid to infections caused by organisms acquired from the patient's endogenous flora (Dancer, 2014). Respiratory tract and enteral colonization with GNB had been associated with infections in critically ill patients (Sertaridou *et al*., 2015). A large percentage of patients colonized by MDR- GNB are not recognized by clinical cultures but are only detected by performing surveillance cultures (Ridgway *et al*., 2014).

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Materials and Methods

Study area. This prospective cross-sectional study was conducted over a period of 2 months from November to December 2018 on patients admitted to ICU of a private Hospital in Alexandria.

Sampling. Throat swabs were taken on admission for the detection of colonization. These patients were further tracked for the development of infections then clinical samples were collected from infected patients according to the site of infection, urine samples (from indwelling catheters), lower respiratory tract infection (sputum from endotracheal tube (ETT) or mini broncho-alveolar lavage (mini-BAL), swabs or aspirates from infected wounds and blood samples. (If infections developed after 48 h, they were considered HAIs). Also, weekly environmental samples were obtained during the studied period, while the patient occupied the partition from bed rails (B.R), bed-side table (B.T), monitor touch pad (M.T.P), nurse call button (N.B), drawer handle (D.H), infusion pump (I.P) (if present), ventilator (V) (if present), floor (F) on both sides of the patient's bed (an area of approximately 10 cm^3), sink (S) (if present).

Ethics approval. The study protocol was approved by Ethical Committee at the High Institute of Public Health (HIPH).

Laboratory investigation. Samples from newly admitted patients were inoculated on both blood agar (BA) and MacConkey's agar plates. Urine samples were cultured on MacConkey's agar plate using sterile disposable loop of 10 µ to obtain countable colonies. Samples from lower respiratory tract infection, swabs and aspirates from infected wounds were inoculated on BA, MacConkey's agar and chocolate agar (CA) plates. Blood cultures were incubated in BACTEC™ Automated Blood Culture System.

Environmental samples: Swabs were incubated overnight in brain heart infusion broth at 37°C. Broth was then subcultured on MacConkey's agar plates. Identification was done according to standard microbiological procedure (Tille, 2013). All gram-negative bacterial isolates recovered from colonized and infected patients as well as those recovered from ICU environment except for the only *Burkholeria cepacia* and some of Acb complex isolates were subjected to antimicrobial susceptibility testing using Kirby Bauer disc diffusion method. (Clinical and Laboratory Standards Institute, 2020)

Results and Discussion

None of the studied patients were infected on admission. The causes of admission were ischemic heart disease, stroke and cerebral hemorrhage.

Table (1, 2 and 3) and Fig. 1 showed that only 10 (6.06%) out of 165 admitted patients were colonized by GNB. Among these colonized patients, 5 were colonized by *K. pneumoniae*, 3 by *E. coli* and 2 by *P. aeruginosa*. Fifty percent of the colonizing isolates were MDR.

None of the isolates caused infections were related to those recovered from colonized patients on admission. Out of 165 patients, 27 (16.365) developed infections. 9 of these infections developed after 48 h and 18 developed in less than 48 h.

Table (4 and 5) and Fig. 2 showed that about 36 GNB isolates were recovered from infected patients, 86.11% of them were MDR.

About 75.00% of patients colonized by MDR GNB were discharged and 25% died, while all patients infected by non MDR GNB were discharged.

Table (6) showed that the rate of environmental contamination due to GNB during the studied period (8 weeks) was 27.06% and about 58.08% of these environmental GNB isolates were MDR. The most contaminated site with gram-negative isolates was the floor (52.94%) followed by sinks (50.00%), bed rails (37.50%), bed-side tables (33.09%), drawer handles (22.06%), infusion pumps (15.42%), ventilators (14.29%) and the least contaminated site was nurse call buttons (9.56%).

Table 1. Follow-up of the 165 studied patients according to occurrence of infection

Patients	No.	%
Infected	27	16.36
Urinary tract infections	21	12.73
Pneumonia	6	3.64
Skin infections	4	2.42
Not infected	138	83.64
Total	165	100.00

Table 2. Follow-up of the 165 studied patients according to onset of infection

Duration of stay	Patients				Total	
	Infected		Not infected			
	No	%	No	%	No	%
≤48 h	18	34.62	34	65.38	52	31.52
48 h-≤1 week	4	4.55	84	95.45	88	53.33
1 week - 1 month	5	20.00	20	80.00	25	15.15
Total	27	16.36	138	83.64	165	100.00
χ^2(p)		21.880*(<0.001*)				

Table 3. Percentages of antibiotic resistance of the 10 gram-negative isolates recovered from colonized patients

Gram-negative Isolates (10)	AMC	CAZ	PRL	TZP	CIP	LEV	AK	CN	IPM	MEM	ETP	CT	TGC
K. pneumoniae (5)	100.00	0.00	NA	NA	80.00	80.00	60.00	40.00	40.00	40.00	60.00	0.00	0.00
E. coli (3)	66.70	33.30	NA	NA	33.30	33.30	33.30	33.30	0.00	0.00	0.00	0.00	0.00
P. aeruginosa (2)	100.00	50.00	0.00	0.00	50.00	50.00	50.00	0.00	0.00	0.00	NA	0.00	NA

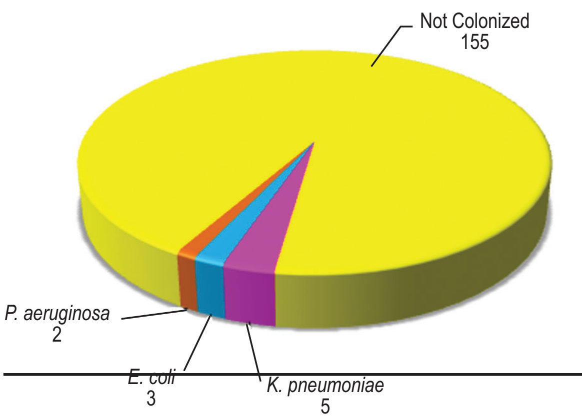

Fig. 1. Distribution of gram-negative bacteria with regard to throat colonization among the 165 studied patients on admission.

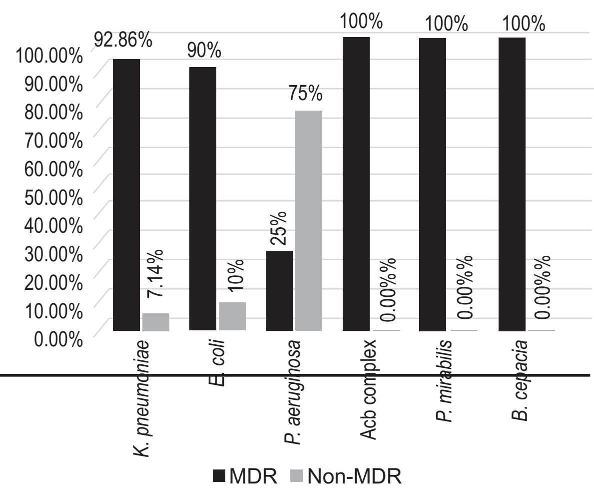

Fig. 2. Distribution of gram-negative isolates recovered from infected patients.

K. pneumoniae was the predominant isolate recovered from both colonized and infected patients (50.00% and 38.89% respectively) as well as from environmental samples (48.29%). *E. coli* and Acb complex represented the two most predominant gram-negative isolates recovered from infected patients second to *K. pneumoniae* (27.78% and 13.89% respectively). Acb complex and *P. aeruginosa* were the two most predominant gram-negative isolates recovered from environmental samples second to *K. pneumoniae* (22.05% and 20.15% respectively) show in Fig. 3.

Figure (4) showed that the highest isolation of GNB were in the first week and there was a marked gradual decrease in GNB isolation on the subsequent weeks except for week 5.

ESBL production was predominant among *K. pneumoniae* isolates from all sample types. It was detected among 40% of isolates recovered from colonized patients, 42.86% from infected patients and 16% from environmental samples. Carbapenem resistance rate was also higher among *K. pneumoniae*

Table 4. Percentages of the antibiotic resistance of the recovered 36 gram-negative isolates from 27 infected patients

Gram-negative Isolates (36)	AMC	CAZ	FOX	FEP	PRL	TZP	CIP	LEV	AK	CN	IPM	ETP	TGC	SXT	TE
K. pneumoniae (14)	85.7	64.3	92.9	78.6	N.A	57.1	85.7	85.7	50.0	71.4	35.7	50.00	0.0	85.7	100.0
E. coli (10)	60.0	60.0	90.0	70.0	N.A	30.0	80.8	80.8	20.0	80.0	20.0	20.0	0.0	70.0	100.0
P. aeruginosa (4)	100.0	25.0	N.A	25.0	0.0 0	0.0	25.0	25.0	0.0	0.0	0.0	N.A	N.A	N.A	N.A
Acb complex (5)	100.0	100.0	N.A	100.0	N.A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	N.A
P. mirabilis (2)	100.0	50.0	100.0	0.00	N.A	N.A	50.0	50.0	0.00	50.0	0.0	50.0	N.A	100.0	100.0
B. cepacia (1)	100.0	100.0	N.A	100.0	100.0	N.A	N.A	100.0	100.0	100.0	100.0	N.A	0.00	0.0	N.A

Table 5. Distribution of MDR gram-negative bacteria among the 36 recovered isolates from 27 infected patients

Isolates	MDR		Not MDR		Total	
	No	%	No	%		
K. pneumoniae	13	92.86	1	7.14	14	38.89
E. coli	9	90.00	1	10.00	10	27.78
P. aeruginosa	1	25.00	3	75.00	4	11.11
Acb complex	5	100.00	0	0.00	5	13.89
P. mirabilis	2	100.00	0	0.00	2	5.55
B. cepacia	1	100.00	0	0.00	1	2.78
Total	31	86.11	5	13.89	36	100.00
χ^2(^{MC}p)		9.406* (0.042*)				

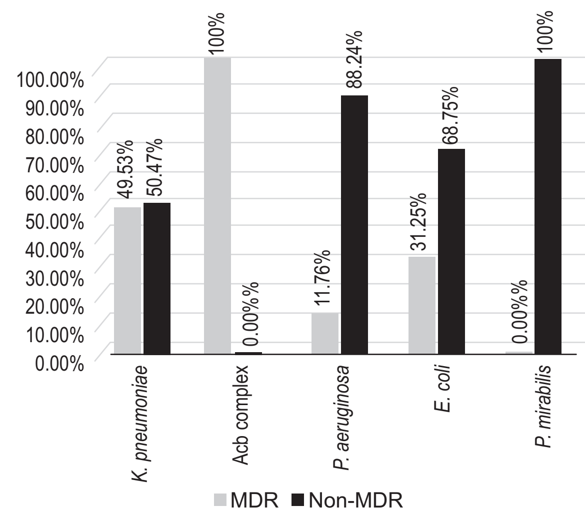

Fig. 3. Distribution of gram-negative isolates from environmental samples.

isolates representing 35.71%, 32.7% from infected patients and environmental samples, respectively and among 20% of *E. coli* isolates recovered from infected patients. Acb complex isolates from all sample types exhibited extremely high levels of resistance to all antibiotics with the exception of colistin and tigecyclin.

No similarity was detected among the same gram-negative isolates recovered from colonized and infected patients.

Table (7) shows comparison between isolates from infected patients and their occupied environment, where 36.11% of isolates recovered from infected patients were the same isolates recovered from their occupied environment. Out of these isolates, *K. pneumoniae* recorded the highest percentage (61.54%) followed by Acb complex (30.77%) and *E. coli* (7.69%). No similarity were recorded among *P. aeruginosa*, *P. mirabilis* or *B. cepacia*.

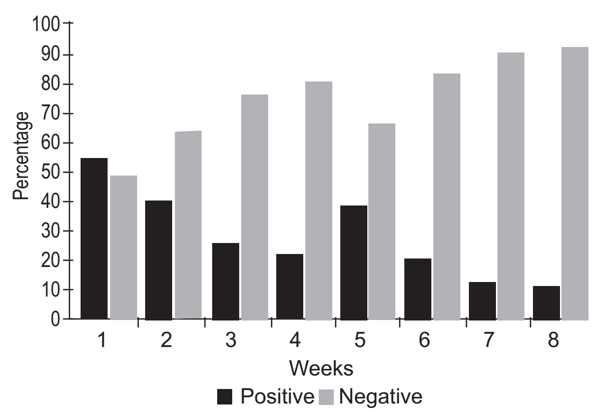

Fig. 4. Isolation of GNB from environmental samples by week of sampling.

Table 6. Distribution of the recovered 263 gram-negative environmental isolates according to collection site

Collection site	Isolated organism											
	P. aeruginosa		*E. coli*		*K. pneumoniae*		Acb complex		*P. mirabilis*		Total (n=263)	
	No	%	No	%	No	%	No	%	No	%	No	%
B.R	9	16.98	4	18.18	27	21.2	9	15.5	2	66.67	51	19.39
B.T	10	18.87	2	9.09	18	14.1	15	25.8	0	0.00	45	17.11
M.T.P	7	13.2	2	9.09	3	10.2	5	8.62	0	0.00	17	6.46
N.B	7	13.2	0	0.00	3	10.2	3	5.17	0	0.00	13	4.94
D.H	4	7.69	1	4.55	17	13.3	8	13.7	0	0.00	30	11.41
I.P	4	7.69	0	0.00	5	3.94	6	10.3	0	0.00	15	5.70
V.	0	0.00	0	0.00	1	0.79	3	5.17	0	0.00	4	1.52
F.	5	9.43	13	59.09	46	36.2	8	13.7	0	0.00	72	27.38
S.	7	13.2	0	0.00	7	5.51	1	1.72	1	33.33	16	6.08

Table 7. Comparison of isolates from patients and their occupied environment

Patients' isolates	Environmental isolates				Total	
	Yes		No			
	No	%	No	%	No	%
K. pneumoniae	8	61.54	6	26.09	14	38.89
E. coli	1	7.69	9	39.13	10	27.78
P. aeruginosa	0	0.00	4	17.39	4	11.11
Acb complex	4	30.77	1	4.35	5	13.89
P. mirabilis	0	0.00	2	8.69	2	5.55
B. cepacia	0	0.00	1	4.35	1	2.78
Total	13	36.11	23	63.89	36	100.00
χ^2(^{MC}p)		12.380*(<0.001*)				

Centers for Disease and Control Prevention (CDC) had used the terms "catastrophic consequences" to declare the progressive development and dissemination of antibiotic resistance (CDC, 2019). MDR-GNB colonization is considered an important risk factor for infection especially in ICUs. Wang *et al*. (2009) from China found that 35.1% of patients were colonized by GNB in their oropharynx. In 2016 Fouda *et al*. reported that in Egypt found that 33.7% of admitted patients were colonized by ESBL isolates and 13% were colonized by MDR-GNB. In the present study, only 6.06% (10 patients) yielded gram-negative bacterial growth from their throat swabs, this very low rate of colonization may be attributed to that the majority of patients (60.61%) were on antibiotics on admission (prior to sampling). Furthermore, obtaining rectal swabs from admitted patients would have been ideal and increase the opportunity for the detection of MDR-GNB colonization especially among Enterobacteriaceae. Regarding the most common colonizing bacteria, Wang *et al*. (2009) from China and Fouda *et al.* (2016) from Egypt found that *K. pneumoniae* and *E. coli* were predominant isolates in their studies which is corresponding to the finding of the present study.

The present study and other similar studies showed the predominance of *K. pneumoniae* among infected patients (Hassan *et al*., 2019; Talaat *et al*., 2016). *E. coli* was second to it in the present study and the studies by Hassan *et al.* (2019) and Talaat *et al.* (2016) from Egypt. The predominance of these organisms may be attributed to the fact that Enterobacteriaceae are ubiquitously found in nature and can colonize medical devices and health care environment (Podschun *et al*., 2001). However, Ibrahim (2018) from Saudi Arabia recorded *Acinetobacter* spp. which is predominant and isolate in their studies.

Talaat *et al*. (2016) also found that the rate of ESBL-producing *Klebsiella* spp. was 42.5% among infected patients, which is very close to the result of the present study in which 6 out of the 14 *K. pneumoniae* isolates accounting for 42.86% from infected patients were ESBL. It should be noted that 40% of the colonizing *K. pneumoniae* isolates also yielded ESBL isolates. This result was also corresponding to another recent Egyptian study by Galal *et al*. (2019) who showed that ESBL producers are widely spread among infected patients. However, these rates were higher than the rates reported by Gupta *et al*. (2019) in the US (12.6%). Moreover, carbapenem resistance in the present study was detected in 35.71% of the *K. pneumoniae* and 20% of *E. coli* isolates recovered from infected patients. Similar finding was recorded in a surveillance study in Egypt by Kotb *et al*. (2020) from 2011 to 2017.

Among the GNB recovered from infected patients in the present study, 86.11% were found to be MDR. Similar studies from Saudi Arabia, India and Egypt also recorded high rates of MDR among gram-negative isolates from infected patients in ICUs ranging from 67.9% to 93.6% (Khalifa *et al*., 2019; Ibrahim, 2018; Moolchandani *et al*., 2017). Inadequate antibiotic use is considered an important pre-disposing factor for the increased prevalence of drug resistance.

The inanimate hospital environment can serve as a reservoir for many GNB that have the ability to survive and infect patients. In the present study, the rate of environmental contamination due to gram-negative isolates during the studied period (8 weeks) was 27.06%, this rate was corresponding to that found by Mehraban *et al*. (2016) from Iran who detected that GNB accounted for about 21% and was lower than that detected by Chaoui *et al*. (2019) from Morocco. This variation from one institution to another and within the same establishment may be attributed to the services, patients, care and techniques practiced.

In the present study, as in patients, *K. pneumoniae* represents the predominant gram-negative isolate from environmental samples accounting for 48.29% followed by Acb complex (22.05%) and *P. aeruginosa* (20.15%). This finding was corresponding to that recorded by Ahmed *et al.* (2019) in a study from upper Egypt. On the other hand, Mehraban *et al*. (2016) found that non-fermentative bacteria including *P. aeruginosa* and Acb complex accounted for the highest rates of environmental gram-negative isolates from ICUs.

In the present study, ESBL production among environmental *K. pneumoniae* and *E. coli* isolates was detected in 16% and 25% respectively which is lower than those detected from colonized and infected patients. Manel *et al*. (2014) in a study from Algeria recorded the prevalence of ESBL production among 28.45% of environmental *K. pneumoniae* and in 25.41% among *E. coli* isolates. The low rate of ESBL isolates recovered from environmental samples may be attributed to effective infection control measures.

The study by Manel *et al*. (2014) showed carbapenem resistance among only 2.51% of Enterobacteriaceae, which was lower than detected among *K. pneumoniae* and *E. coli* environmental isolates in the present study (32.7% and 6.25% respectively). This high rate of carbapenem resistance among environmental *K. pneumoniae* isolates is very close to those recovered from infected patients (35.7%). It should be noted that, despite gradual decrease in the number of carbapenem resistant *K. pneumoniae* isolates recovered from the ICU environment during the study period, these isolates in particular stayed in the ICU environment for up to the 7th week of the study. This may be due to the higher tendency of these MDR organisms to form biofilm which enables them to survive on dry hospital surfaces for a long time with an increased resistance towards inactivation by disinfectants (Vickery *et al*., 2012).

Comparison between the same isolates recovered from infected patients and their occupied environment, with higher rates among *K. pneumoniae* and Acb complex (61.54% and 30.77% respectively), suggest a cross contamination between infected patients and their surrounding environment and demonstrate the importance of the hospital environment in the dissemination of HAIs (Shimose *et al*., 2016).

As part of the conventional strategy for hand hygiene in any hospital, sinks are placed in nearly all patient rooms and hospital wards, Although sinks nearby patients are regarded as a best ICU design, these sinks has been reported as a disseminating factor for HAIs as early as the 1970s (Hopman *et al*., 2017). The present study showed high contamination rates of sinks by GNB accounting for 50%, with the predominance of *P. aeruginosa* and *K. pneumoniae* accounting for 7 isolates for each out of the 16 positive gram-negative isolates. This may be attributed to the aqueous environment of sinks which enhance biofilm formation by attaching to and colonizing solid surfaces. Similar results were recorded by Gordon *et al.* (2017) in a systemic review with data from Europe, Asia, north America and Australia who recorded *P. aeruginosa* and *K. pneumoniae* among the most frequently isolates from water-related environment in ICU.

Conclusion

Low HAIs rate was detected in the ICU, with the predominance of *K. pneumoniae* isolates. High rate of MDR-GNB was detected among all gram-negative isolates. High rate of cross contamination between infected patients and their occupied environment was detected among *K. pneumoniae* and Acb complex isolates. No similarity was shown between colonized and infection causing isolate.

Conflict of Interest. The authors declare that they have no conflict of interest.

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