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ORIGINAL ARTICLE

Prevalence and Antibiotic Resistance Patterns of Multidrug-Resistant (MDR) Bacteria Isolated from Pediatric Intensive Care Units

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ABSTRACT

Background: *The escalating prevalence of antibiotic-resistant organisms, coupled with limited discovery efforts of novel drugs, poses a critical challenge. Particularly pediatrics are at an elevated risk, making periodic updates on antibiotic resistance data essential.* ***Objective:*** *This study aimed to determine the current prevalence of MDR bacteria in different PICUs and analyze the distribution of MDR infections across different childhood stages at a specialized University hospital in Cairo. Furthermore, we aim to detect the carbapenemase encoding genes in the CRE strains with the goal of highlighting the increasing rates of MDR infections especially in pediatric health care centers and increase awareness towards antibiotics usage.* ***Methodology:*** *In this study, 455 clinical samples were obtained from patients who were admitted to various pediatric ICUs. Samples were identified and tested for susceptibility to different antibiotics. Additionally, Enterobacteriaceae samples which had shown a potential carbapenemase activity were tested using multiplex PCR assay.* ***Results****: Our Results revealed that out of 318 bacterial samples, 290 (91%) were found to be MDR strains, 84 (92%) out of 91 Gram-positive isolates were MDR while out of 227 Gram-negative isolates 206 (90%) were MDR. Noticeably, neonates and toddlers are the most vulnerable age groups to the MDR infections with the percentage of 96.87% (31/32) and 95.5% (43/45), respectively.* ***Conclusion****: The study highlights the extensive prevalence of MDR bacteria in PICUs, emphasizing the urgent need for robust infection control measures and antibiotic stewardship programs.*

INTRODUCTION

The effectiveness of antibiotics in combatting disease-causing microorganisms stands as one of the crowning achievements of modern medicine. Over the past two decades, the expansions of resources and significant technological advancements have led to a substantially increased availability of drugs in developing nations. Consequently, a considerably greater number of people are now receiving essential treatments compared to just twenty years ago. Unfortunately, this positive progress is marred by the absence of regulatory policies governing antibiotic prescriptions, resulting in misuse. This, in turn, has diminished the efficacy of many antimicrobial agents, and various pathogens are increasingly showing resistance [1].

Resistance to one antibacterial class can usually be achieved through multiple pathways, and single bacterial cell may be capable of using a group of mechanisms of resistance to survive the effect of an antibiotic. Resistance to antibiotics is typically the result of antibiotic destruction or modification, changes in target sites, and reduced antibiotic accumulation due to either decreased permeability or increased efflux.

β- Lactamases are the best example of antibiotic resistance mediated by antibiotic destruction, they are the enzymes responsible for the destruction of β-lactam antimicrobial compounds by destroying the amide bond of the β-lactam ring, causing the ring to open and rendering the antimicrobial ineffective. Among these, ESBLs and AmpC enzymes which are a subgroups that are responsible for resistance to extended-spectrum cephalosporins and penicillins. Carbapenemases, another type of beta-lactamase, represent a more serious concern as they confer resistance to carbapenems, often considered last-resort antibiotics. The emergence of carbapenemase-producing bacteria, such as Klebsiella pneumoniae carbapenemases (KPC) or New Delhi metallo β -lactamases (NDM), further limits treatment options, emphasizing the critical importance of surveillance, infection control, and cautious antibiotic use to control the spread of these resistant enzymes.

A clear and specific terminology of the degree of antimicrobial resistance in bacteria such as multidrug-resistant (MDR) bacteria, extensively drug-resistant

(XDR) bacteria, and pan drug-resistant (PDR) bacteria is more than important. Consequently, The European Centre for Disease Prevention and Control aimed to globally standardize and manage these terms in an article published in 2012 [2]. In this article, a universally standardized terminology for describing resistance profiles across all bacteria was introduced. Multidrug-resistant (MDR) was characterized by acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) indicated non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and pan drug-resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories.

Multiple drug resistance has been observed in a range of pathogens that are more prevalent in Intensive Care Units (ICUs) compared to other sections of the hospital [3]. Furthermore, research has indicated that the longer a patient stays in the hospital, the greater his risk of infection. The median time for acquiring an infection from a MDR pathogen during a hospital stay was found to be 8 days. Each additional day of hospitalization raised the likelihood of contracting an MDR pathogen infection by approximately 5% [4].

Patients in ICU face a heightened risk of contracting infections from MDR organisms, which are more prevalent in this clinical setting. This vulnerability isn't limited to the actual site of infection but also extends to the microbial communities within the digestive tract and other commensal flora. In these environments, the abundant presence of bacteria can facilitate the rapid proliferation of drug-resistant microorganisms. Even seemingly harmless bacteria can transfer these resistance genes to pathogenic counterparts, either directly through DNA exchange via conjugation or via extra-chromosomal plasmid DNA.

MDR bacteria can be found in pediatric and neonatal hospitals, particularly within the ICUs. Children admitted to the Pediatric ICU face an elevated risk of MDR organism infections due to the frequent use of invasive medical devices and the need for continuous parenteral nutrition during their ICU stays in addition to their compromised immune systems[5]. Research indicates that neonatal patients, in particular, are more prone to MDR Gram-negative bacteremia, resulting in shorter hospital stays but higher mortality rates [6].

Antibiotic use has unfavorable effects that go beyond the emergence of resistance in the organisms it is intended to treat. From 2011 to 2015, an analysis of pediatric data collected by the National Electronic Injury Surveillance System revealed that antibiotics were responsible for nearly 50% of emergency department visits related to adverse effects caused by systemic medications [7].

According to a study conducted in a University hospital in Egypt [8] , MDR organisms were not limited to healthcare facilities. More alarming was the discovery of XDR bacteria in cases of community-acquired infections. In this later study, it was reported that among cases of resistant bacteria acquired in the PICU, 61.1% were MDR, 30.56% were XDR, and 5.56% were PDR. Meanwhile, in the community acquired infections, MDR bacteria accounted for 56.25% of cases, with the emergence of XDR bacteria at 6.25%, a relatively high figure. This increase in resistance has been linked to the widespread availability of antibiotics as over-the-counter medications. Clearly, the inappropriate and extensive use of antibiotics in Egypt has contributed to antibiotic resistance, which, in turn, impacts the quality of patient care through increased morbidity and mortality rates and substantial economic repercussions. Therefore, this was the impetus to carry out the current study to assess and scrutinize the bacterial resistance profiles in ICUs in some Egyptian hospitals.

METHODOLOGY

This observational cross-sectional study was conducted over the period from February 2023 to July 2023, Samples were obtained from all pediatric patients who stayed more than 48 h in ICU with clinical symptoms or signs of Hospital acquired infection (HAI). Ages are varied from one day old to fourteen years old and both sexes were included.

This study was revised and approved by the Ethical Review Committee of Helwan University (Approval no. 06H2023) and consent was obtained from all legal guardians of the patients.

Specimen collection, transport and storage:

A total of 455 clinical samples were collected, including specimens from wounds, sputum, stool, ascetic fluid, endotracheal aspirates (ETA), pus, urine, and blood. All samples collected were subjected to microbiological procedures following the standard protocols for each specimen type. All the samples were transported within 15 min to the microbiology laboratory and stored at 4°C until needed.

Cultivation of the specimens:

Blood and ascitic fluid specimens were first detected by BACTEC blood culture systems to confirm or rule out possibility of infection, then subculture from the positive bottle was done.

Different clinical specimens including blood and ascitic fluid specimens were cultivated on MacConkey's agar, blood agar and chocolate agar. Urine samples were also cultivated onto cysteine lactose electrolyte deficient agar (CLED). For samples such as ascetic fluid or pus samples, specimens were inoculated in anaerobic glass jar for the growth of anaerobic pathogens.

Identification of bacterial isolates

1. Direct film examination of isolates by using conventional gram staining techniques for fast primary detection of pathogens.

2. Identification of isolates using MALDI-TOF MS throughout bioMerieux VITEK MS system

Antibiotic sensitivity testing:

All bacterial isolates underwent antibiotic susceptibility testing through BIOMÉRIEUX VITEK®2 compact system and the modified Kirby-Bauer disc diffusion technique (CLSI, 2022).

Screening for carbapenemase producing Enterobacteriaceae:

Given that nearly 50% of pathogenic infections occurring in the ICUs were caused by members of the Enterobacteriaceae family, and considering the crucial role of carbapenems as the final line of defense against challenging MDR bacterial infections and frequently administered in Pediatric ICUs, it is essential to investigate into carbapenem resistance within the Enterobacteriaceae family.

All Enterobacteriaceae isolates were assessed for their susceptibility to three different carbapenem antibiotics namely, imipenem, meropenem, and ertapenem. The isolates that showed intermediate or resistant zones for either one or all of the previously-mentioned antimicrobial agents, it indicated potential for carbapenemase production and therefore, multiplex PCR assays were used to ascertain the diagnosis.

Genotypic detection of carbapenemase producing Enterobacteriaceae:

Specific primers designed for the detection of the five major families of carbapenemases were used (VIM, IMP, NDM, OXA-48 and KPC) and described in table 1.

Table 1: description of the designed primers

Target	Ambler class	direction	Nucleotid sequences (5'-3')	Amplicon length (bp)
bla_{VIM}	Class B	Forward	GATGGTGTTTGGTCGCATA	390
		Reverse	CGAATGCGCAGCACCAG	
bla_{IMP}	Class B	Forward	GGAATAGAGTGGCTTAAYTCTC	232
		Reverse	GGTTTAAYAAAACAACCACC	
bla_{NDM}	Class B	Forward	GGTTTGGCGATCTGGTTTTC	621
		Reverse	CGGAATGGCTCATCACGATC	
bla_{OXA-48}	Class D	Forward	GCGTGGTTAAGGATGAACAC	438
		Reverse	CATCAAGTTCAACCCAACCG	
bla_{KPC}	Class A	Forward	CGTCTAGTTCTGCTGTGTTG	798
		Reverse	CTTGTCATCCTTGTTAGGCG	

DNA were extracted using the boiling method. Two different multiplex reactions were prepared. The first multiplex reaction comprises Taq master mix, Nuclease free water, NDM-Forward, NDM-Reverse, OXA-48-Forward, OXA-48-Reverse, KPC-Forward, KPC-Reverse and Target DNA. The second multiplex reaction contained Taq master mix, Nuclease free water, VIM-Forward, VIM-Reverse, IMP-Forward, IMP-Reverse and Target DNA.

The amplification was performed using the thermal cycler and the PCR cycling profile was adjusted to give the following thermal conditions:

1. Initial denaturation: 94°C for 5 min
2. 30 cycles of Denaturation: 94°C for 30 sec, annealing: 60°C for 50 sec and Extension: 72°C for 1 min
3. Final extension: 72°C for 7 min
4. Maintenance: 4°C

For Detection of PCR amplified product Agarose gel electrophoresis and ethidium bromide are used for visualizing the PCR product.

RESULTS

Results of identification:

Out of the 455 clinical samples 318 turns out to be pathogenic bacterial infections while the remaining 137 were categorized as normal flora or fungal infections and were subsequently disregarded. Among these 318 isolates, 227 (71.3%) were Gram-negative bacteria and 91 (28.6%) were Gram-positive bacteria. *Klebsiella* spp. represented the majority of isolates 122/318 (38.3%) followed by *CoNS* 59/318 (18.5%), *Pseudomonas aeruginosa* 32/318 (10%), *Acinetobacter baumannii* 30/318 (9.4%), *Staphylococcus aureus* 17/318 (5.3%) and *E.coli* 15/318 (4.7%)

Results of Antibiotic susceptibility testing:

Antibiotic susceptibility testing was conducted for all the bacterial isolates. MDR bacteria are characterized by their resistance to at least one antimicrobial agent in three or more distinct antimicrobial categories. the antibiotic susceptibility testing has revealed that out of 318 bacterial isolates, 290 (91%) were found to be MDR strains and 84 (92%) out of 91 Gram-positive isolates were MDR while out of 227 Gram-negative isolates, 206 (90%) were MDR. linezolid, tigecycline, and vancomycin exhibit the highest antimicrobial activity against Gram-positive infections, while penicillins and cephalosporins demonstrated the least effectiveness. Regarding Gram-negative susceptibility, tigecycline demonstrated the highest efficacy among all other drugs, followed by imipenem, gentamycin, and amikacin. On the contrary, penicillins, as well as first, second, and third-generation cephalosporins, proved to be the least effective drugs. The Distribution of MDR infection among different bacterial strains is shown in table 2.

Table 2: Distribution of MDR infection among different bacterial strains

	No. of MDR	No. of susceptible	Total no.	% of MDR
Klebsiella spp.	116	6	122	95.08%
CONS	56	3	59	94.91%
Pseudomonas aeruginosa	30	2	32	93.75%
Acinetobacter baumannii	29	1	30	96.55%
Staphylococcus aureus	16	1	17	94.11%
E. coli	7	8	15	46.66%
Stenotrophomonas maltophilia	11	0	11	100%
Enterococcus spp.	8	2	10	80%
Chryseobacterium indologenes	3	0	3	100%
Serratia marcescens	1	2	3	33.33%
Streptococcus spp.	3	0	3	100%
Enterobacter spp.	3	0	3	100%
Burkholderia spp.	3	0	3	100%
Morganella morganii	0	2	2	0%
Proteus mirabilis	1	0	1	100%
Corynebacterium amycolatum	1	0	1	100%
Rothia mucilaginosa	0	1	1	0%
Achromobacter denitrificans	1	0	1	100%
Citrobacter freundii	1	0	1	100%
Total	**290**	**28**	**318**	**91.19%**

Susceptibility testing results for Enterobacteriaceae, Gram-negative and Gram-positive isolates are shown in tables 3, 4 and 5, respectively.

Table 3: Susceptibility testing results for Enterobacteriaceae isolates

	klebsiella spp.		***E. coli***		***S. marcescens***		***Enterobacter spp.***		***M. morganii***		***C. freundii***		***P. mirabilis***	
total	122		15		3		3		2		1		1	
	R	R%	R	R%	R	R%	R	R%	R	R%	R	R%	R	R%
AMC	115	94	10	66.7	-	-	-	-	-	-	-	-	0	0
AMS	120	98	10	66.7	-	-	-	-	0	0	1	100	0	0
TPZ	106	87	11	73.3	1	33.3	3	100	0	0	1	100	0	0
CFZ	121	99	13	86.7	-	-	-	-	-	-	-	-	0	0
FOX	111	91	10	66.7	-	-	-	-	0	0	1	100	1	100
CAZ	116	95	12	80	1	33.3	3	100	0	0	1	100	1	100
CTX	114	93	12	80	1	33.3	3	100	0	0	1	100	1	100
CTR	111	91	10	66.7	1	33.3	3	100	0	0	1	100	1	100
FEP	105	86	10	66.7	0	0	2	66.7	0	0	1	100	1	100
CRO	117	96	-	-	-	-	3	100	-	-	-	-	0	0
CFX	109	89	8	53.3	1	33.3	3	100	-	-	-	-	1	100
SXT	106	87	8	53.3	1	33.3	3	100	0	0	1	100	1	100
CIP	94	77	4	26.7	1	33.3	3	100	0	0	1	100	1	100
Lev	97	80	5	33.3	1	33.3	3	100	0	0	1	100	1	100
IPM	80	66	1	6.67	0	0	3	100	0	0	1	100	0	0
MEM	90	74	3	20	0	0	3	100	0	0	1	100	0	0
ETP	86	70	1	6.67	0	0	3	100	0	0	1	100	0	0
Gen	82	67	2	13.3	0	0	2	66.7	0	0	1	100	0	0
AmK	87	71	1	6.67	0	0	3	100	0	0	1	100	0	0
TGC	39	32	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Susceptibility testing results for Gram-negative isolates

	P. aeruginosa		***A. baumannii***		***S. maltophilia***		***C. indologenes***		***Burkholderia spp.***		***A. denitrificans***	
total	32		30		11		3		3		1	
	R	R%	R	R%	R	R%	R	R%	R	R%	R	R%
AMC	-	-	-	-	11	100	-	-	-	-	1	100
AMS	-	-	28	93.3	11	100	3	100	-	-	1	100
TPZ	26	81.25	27	90	11	100	2	66.7	3	100	1	100
CFZ	-	-	30	100	11	100	-	-	3	100	1	100
FOX	31	96.8	30	100	11	100	2	66.7	3	100	1	100
CAZ	29	90.6	30	100	11	100	3	100	2	66.7	1	100
CTX	-	-	30	100	11	100	3	100	2	66.7	1	100
CTR	-	-	28	93.3	11	100	3	100	1	33.3	0	0
FEP	25	78.1	26	86.7	11	100	1	33.3	1	33.3	1	100
CRO	31	96.8	30	100	11	100	-	-	3	100	1	100
CFX	28	87.5	30	100	11	100	-	-	3	100	1	100
SXT	-	-	24	80	6	54.5	0	0	0	0	1	100
CIP	29	90.6	24	80	8	72.7	3	100	2	66.7	1	100
LEV	30	93.7	27	90	8	72.7	3	100	2	66.7	1	100
IPM	27	84.3	20	66.7	11	100	1	33.3	3	100	0	0
MEM	26	81.2	22	73.3	11	100	2	66.7	3	100	0	0
ATM	26	81.2	-	-	8	72.7	-	-	-	-	-	-
Gen	29	90.6	24	80	10	90.9	3	100	3	100	0	0
AMK	28	87.5	24	80	8	72.7	3	100	3	100	0	0
TGC	-	-	7	23.3	1	9.1	1	33.3	1	33.3	1	100

Table 5: Susceptibility testing results for Gram-positive isolates

	CONS		***S. aureus***		***Enterococcus spp.***		***Streptococcus spp.***		***C. amycolatum***		***R. mucilaginosa***	
total	59		17		10		3		1		1	
	R	R%	R	R%	R	R%	R	R%	R	R%	R	R%
AMC	56	94.9	16	94.1	8	80	3	100	0	0	0	0
AMS	56	94.9	16	94.1	8	80	3	100	0	0	0	0
TPZ	56	94.9	16	94.1	8	80	3	100	0	0	0	0
CFZ	56	94.9	16	94.1	-	-	3	100	1	100	0	0
FOX	56	94.9	16	94.1	-	-	3	100	1	100	0	0
CAZ	56	94.9	16	94.1	-	-	3	100	0	0	0	0
CTX	56	94.9	16	94.1	-	-	3	100	0	0	0	0
CTR	56	94.9	16	94.1	-	-	3	100	1	100	0	0
FEP	56	94.9	16	94.1	-	-	3	100	0	0	0	0
CFX	56	94.9	16	94.1	-	-	3	100	0	0	0	0
SXT	42	71.1	2	11.7	-	-	-	-	-	-	-	-
CIP	39	66.1	12	70.5	5	50	1	33.3	1	100	0	0
LEV	39	66.1	14	82.3	5	50	1	33.3	1	100	0	0
IPM	56	94.9	16	94.1	-	-	2	66.7	0	0	0	0
MEM	56	94.9	16	94.1	-	-	2	66.7	0	0	0	0
GEN	24	40.6	1	5.8	3	30	1	33.3	0	0	0	0
TGC	6	10.1	3	17.6	0	0	0	0	0	0	0	0
DOX	21	35.5	8	47	5	50	0	0	0	0	0	0
VAN	8	13.5	2	11.7	6	60	0	0	0	0	0	0
LZD	4	6.7	0	0	0	0	-	-	-	-	-	-
DA	36	61	9	52.9	-	-	1	33.3	1	100	0	0
ER	45	76.2	8	47	8	80	2	66.7	0	0	0	0

AMC, amoxicillin/clavulanate; AMS, ampicillin/sulbactam; TPZ,PIPERCILLIN/TAZOBACTAM; CFZ, Cefazolin; FOX, Cefoxitin; CAZ, Ceftazidime; CTX, Cefotaxime; CTR, Ceftriaxon; FEP, Cefepime; CRO, Cefuroxime; CFS, Cefoperazone/sulbactam; SXT, Cotrimoxazole; CIP, Ciprofloxacin; LEV, Levofloxacin; IPM, Imipenem; MEM, Meropenem; ETP, Ertapenem; ATM, Aztreonam; GEN, Gentamicin; AMK, Amikacin; TGC, Tigecycline; DOX, Doxycycline; VAN, Vancomycin; LZD, Linezolid; DA, Clindamycin; ER, Erythromycin.

Male and female children from the six age groups recommended by the American Academy of pediatrics were included in this study, so we took into consideration the correlation between the age and the liability to MDR infection.

The study revealed that neonates and toddlers were the most vulnerable age groups to MDR infections, with percentages of 96.87% (31/32) and 95.5% (43/45), respectively. Infants were following, showing a rate of 93.85% (107/114). Subsequently, early childhood showed a rate of 86.5% (71/82), middle childhood at 85% (34/40), and adolescence at 80% (4/5)

Results of screening for carbapenemase producing Enterobacteriaceae:

Among the 318 isolates, 147 (46.2%) isolates were from Enterobacteriaceae family. Antibiotic susceptibility testing was performed on all the Enterobacteriaceae isolates for imipenem, meropenem, and ertapenem. If any isolate showed decreased sensitivity to either one or all of these antimicrobial agents, it indicated potential for Carbapenemase production and therefore, genotypic confirmatory test was used to ascertain the diagnosis. The numbers of carpabenem resistance bacteria among Enterobacteriaceae samples are shown in figure 1.

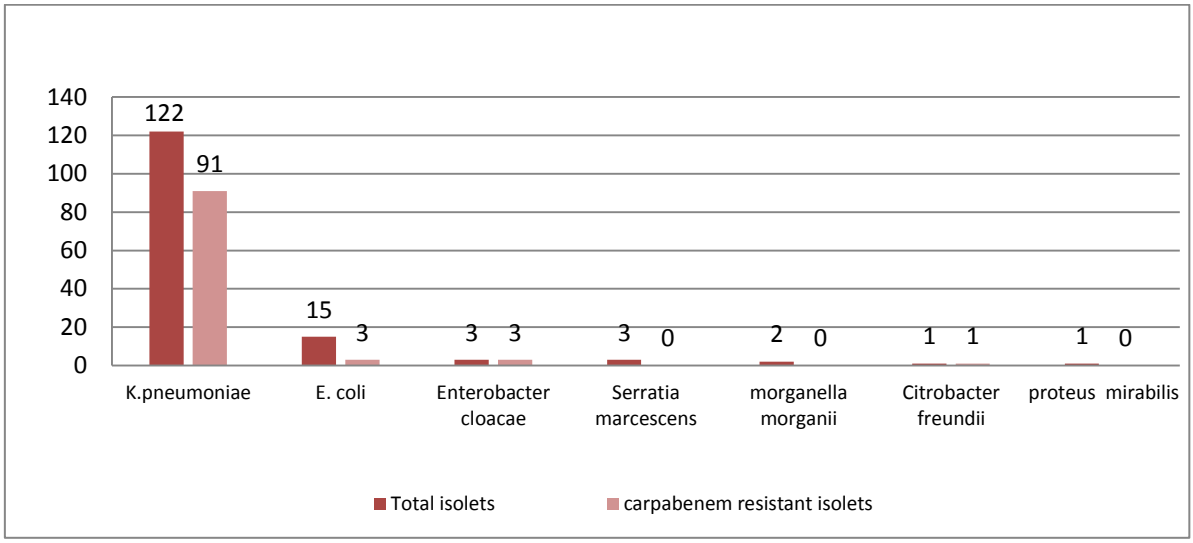

Fig. 1 Distribution of carpabenem resistance among Enterobacteriaceae isolets.

Genotypic confirmatory tests for CPE:

91 *K.pneumoniae* isolates, 3 *E.coli* isolates, 3 *Enterobacter spp.* Isolates and 1 *Citrobacter freundii* isolate were tested for carbapenemase production using multiplex PCR. All isolates were found to be harboring at least one carbapenemase encoding gene. The Distribution of the carbapenemase encoding genes in the CRE strains is shown in table 6.

Table 6: Distribution of the carbapenemase encoding genes in the CRE strains.

	No. of strains	***bla*$_{KPC}$**	***bla*$_{OXA-48}$**	***bla*$_{IPM}$**	***bla*$_{VIM}$**	***bla*$_{NDM}$**	***bla*$_{OXA-48}$ + *bla*$_{NDM}$**	***bla*$_{KPC}$ + *bla*$_{OXA-48}$ + *bla*$_{NDM}$**
K.pneumoniae	**91**	***0***	***0***	***0***	***0***	***5***	***67***	***19***
E. coli	**3**	***0***	***0***	***0***	***0***	***3***	***0***	***0***
Enterobacter spp.	**3**	***0***	***0***	***0***	***0***	***3***	***0***	***0***
Citrobacter freundii	**1**	***1***	***0***	***0***	***0***	***0***	***0***	***0***
total	**98**	***1***	***0***	***0***	***0***	***11***	***67***	***19***

DISCUSSION

In recent times, the growing prevalence of drug-resistant pathogens has sparked worries regarding the effectiveness of conventional antimicrobial agents. This resistance has an impact not only on the therapy of the individual but also on the infection control in the hospitals. A significant feature of medical microbiology in the last decade has been increasing concern about antibiotic resistance. This has led to heightened awareness rates locally, nationally and internationally. Nonetheless, the extent of variation observed across different geographic regions underscores the necessity of acquiring local microbiological data to formulate pertinent recommendations.

In this study 318 pathogenic bacterial isolates were recovered from the examined clinical specimens. More than 35.8% (114/318) of these specimens were isolated from infants with age range within 28 days to 12 month, infancy samples percentage were followed by early childhood samples with percentage of 25.7%(82/318) then toddlers with percentage of 14.15%(45/318), Middle childhood with percentage of 12.57% (40/318), neonate with percentage of 10.06% (32/318) and Adolescence with percentage of 1.57% (5/318).

The results of our study revealed that among the 318 examined specimens 227 were Gram-negative bacteria with the percentage of 71% while the Gram-positive bacteria were only 29% (91/318) of the total tested isolates. Our results were in alignment with Fahim study [9] where Gram-negative isolates were more common, accounting for 57.5% of cases, in contrast to Gram-positive isolates, which made up 31.1% of the total. Similar results also were obtained by Hassan *et al.* [10], in a study done at a tertiary care pediatric Hospital Mansoura, Egypt, where Gram-negative bacteria were recovered from 47.4% of positive cultures, with Gram-positive bacteria accounting for 29.9% of the cases, and (22.7%) were non bacterial infections.

Isolates in the current study were identified by MALDI-TOF MS. This showed that most of the isolates were *K. pneumoniae* (122/318) representing 38.3% followed by CoNS (59/318) representing 18.5%, *Pseudomonas aeruginosa* (32/318) representing 10.06%, *Acinetobacter baumannii* (30/318) representing 9.43%, *Staphylococcus aureus* (17/318) representing 5.34% and *E. coli* (15/318) representing (4.71%). this results was partially in line with Fahim study [9] at Ain Shams University Hospitals in Cairo where The most commonly isolated pathogens included *Klebsiella spp*. (22.5%), *Escherichia coli* (13.4%), and CoNS (12.5%), while *Enterobacter spp., Proteus spp., and Streptococcus spp*. constituted the smallest group among the tested isolates. *Klebsiella spp*. were also the most frequently isolated bacteria as reported by Moustafa *et al.* study [11] which was held at a Pediatric ICU in Alexandria University Pediatric Hospital, Egypt. The latter study aimed to calculate the occurrence or frequency of healthcare-associated infections. Rates among children in PICU of a Tertiary Care Hospital, this study has reported that the most frequently encountered pathogens, constituting 56% of cases, were *Klebsiella spp*., after that come *Acinetobacter spp.* and *Staphylococci spp.* In contrast, a different ordering were reported by Moussally *et al.*[12] in their retrospective study which was done at the American University of Beirut Medical Center and a total of 15,901 isolates were included in their study. The findings of the latter study have revealed that *E. coli* was the most frequently identified (43.7%), followed by *Klebsiella spp*. (15.7%) and *Pseudomonas aeruginosa* (13.7%). However, this dissimilarity can be explained by the different proportions of the clinical specimen types where the majority of clinical specimens were comprised of urine samples (39%) followed by wounds (29%) then respiratory secretions (26%). On the contrary, in the current study urine and wound culture represented the least shares of our clinical specimens.

During the study period, the 318 bacterial isolates yielded 290 MDR isolates with a percentage of 91%. These data are in line with findings from other studies and closely mirrors the research conducted by Ibrahim *et al*.[13] that reported a high percentage of MDR among bacterial isolates from samples collected from 160 pediatric patients admitted to ICUs of Beni-Suef University Hospital. The latter study has reported that MDR bacteria represented 90% (126/140) of total bacterial isolates while only 10% (14/140) were non-MDR organisms. On the other hand, our findings were more optimistic than results of other studies, for instance Moustafa *et al.* [11] who claimed the outcomes of their antimicrobial susceptibility testing revealed that all of the isolates displayed multidrug resistance. This difference could be explained by the limited sample size, as that study only included 16 patients with 25 bacterial infections.

However, in the current study 100 % of the *Stenotrophomonas maltophilia*, *Chryseobacterium indologenes*, *Streptococcus* spp., *Enterobacter* spp., *Burkholderia* spp., *Proteus mirabilis*, *Corynebacterium amycolatum*, *Achromobacter denitrificans* and *Citrobacter freundii* isolates were found to be MDR. Lower percentages of MDR were detected for *Acinetobacter baumannii* 96% (29/30), *Klebsiella spp.* with 95% (116/122), CoNS 94.9% (56/59), *Staphylococcus aureus* 94.1% (16/17), *Pseudomonas aeruginosa* 93.7% (30/32), *Enterococcus spp.* 80% (8/10) and *E. coli* 46.6% (7/15). Once more, our results are in agreement with that reported by Ibrahim *et al*., 2019[13] where 100% of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp*., *Stenotrophomonas maltophilia*, *S. aureus* and *Enterococci spp.* isolates were MDR. While 95.1% and

72.7% of *Klebsiella pneumoniae* and CoNS were found to be MDR, respectively.

Interestingly, a study conducted at Tanta and Mansoura University Hospitals in 2017[14] revealed that *Pseudomonas aeruginosa* displayed a lower level of resistance. In this particular study, just 43.8% of *Pseudomonas aeruginosa* isolates demonstrated MDR. Furthermore, in a separate study conducted in 2021[15], only 82.5% of *K. pneumoniae* isolates exhibited resistance. The findings from these two latest studies are raising the concerns about the alarming progression of drug resistance among various bacterial strains throughout the years.

The current study results regarding the distribution of isolated species among age groups were in line with Kahal *et al*. results [16] as *S. aureus* was more prevalent in early childhood group than any other group, also, *Pseudomonas aeruginosa* was more abundant in the infancy group.

In the present study neonates and toddlers were the most vulnerable age groups to the MDR infections with the percentage of 96.87% (31/32) and 95.5% (43/45), respectively. Infants group comes next, with no huge difference, to the latter two groups with percentage of 93.85% (107/114) followed by early childhood that comes with percentage of 86.5% (71/82), middle childhood with percentage of 85% (34/40) and adolescence with percentage of 80% (4/5). In alignment with our findings, a study conducted on patients in neonatal ICUs at Fayoum University Pediatric Hospital showed that among 379 isolates, 332 (87%) were identified as multidrug-resistant organisms (MDROs) [17].

On the other hand, a recent local study by Seleem *et al*. [18] conducted at Zagazig University children's hospital, reported that infants were more susceptible to MDR infections than neonates as the percentage of MDR infections in infancy group were 60% (12/20) while the percentage in neonates was 50% (47/93). The dissimilarity in results can be attributed to the difference in sample sizes across age groups. In our study, infants constituted the largest proportion at 35.8% of the total samples, whereas neonates made up only 10%. Conversely, in the subsequent study, neonates comprised 62% of all collected samples, with infants accounting for only 13%. However, the available data regarding the correlation between the pediatric age groups and the liability to MDR infections aren't enough.

Regarding antibiotic sensitivity of Gram-positive pathogens, our results showed that linezolid, tigecycline and vancomycin have the highest antimicrobial activity against Gram-positive infections in order, whereas penicillins and cephalosporins were the least effective drugs. For instance from a total of 59 tested CoNS isolates only 6% (4/59), 10% (6/59) and 13% (8/59) were resistant to linezolid, tigecycline and vancomycin, respectively. On the other hand, 94.9 % (56/59) were resistant to all of amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, Cefazolin, cefoxitin, ceftazidime, cefotaxime, ceftriaxone and cefepime. Concerning the Gram-negative susceptibility, tigecycline showed the highest efficacy over all the other drugs followed by imipenem, gentamycin and amikacin, yet again, penicillins, first generation, second generation and third generation cephalosporins were the least effective drugs. In agreement with our results, an Egyptian study held at Mansoura University pediatric Hospital over the course of a year, Hassan *et al*.[10] reported that vancomycin was the most effective antimicrobial agent against Gram-positive infections while amikacin and imipenem were on the top of the antibiotic list of defeating Gram-negative infections. Nazeih *et al*. [19] reported a similar finding in their study where CoNS and *S. aureus* showed a complete sensitivity to vancomycin, linezolid and tigecycline, yet, same samples showed high resistance (95%-100%) against penicillin, cefazolin, ampicillin, cefotaxime and ampicillin-sulbactam. Similarly, Kahal et al., 2023 [16] reported in retrospective cohort study which was done at Damascus Hospital, Syria that the most effective antimicrobial agents against *Pseudomonas aeruginosa* after colistin were amikacin, imipenem and gentamycin. Interestingly, a 3 years epidemiologic study carried out at Maringá University Hospital in Brazil [20] claimed that Klebsiella spp. and Enterobacter spp. isolates showed a higher susceptibility levels to meropenem and levofloxacin , This difference can be explained by the establishment of a multidisciplinary surveillance program aimed at preventing and controlling MDROs. This program provides rapid detection of resistance genes, enabling swift isolation of patients and implementation of essential control measures. These efforts result in achieving a significant reduction in the prevalence of Enterobacteriaceae that were positive for resistance genes.

In order to identify the genes associated with carbapenemase production, a genotypic analysis was done on the Enterobacteriaceae isolates which showed a reduced sensitivity to any of the carbapenems used in the susceptibility testing. Moreover, 66% of Enterobacteriaceae isolates were found to be carbapenemase producers. This was in line with the results of a local study [21] that found a high ratio of carbapenem resistance (58.5%) within the Enterobacteriaceae isolates. Similarly, a high occurrence of carbapenem resistance was reported by Kotb et al., 2020 [22] where out of 1,598 cases of Healthcare-Associated Infections caused by Enterobacteriaceae, 871 cases, representing 54.1%, were resistant to carbapenem antibiotics. Closely looking on the latter study, the ratios of the carbapenem-resistant *Klebsiella spp*. (53%) and *E.coli* (27%) isolates

to the each total number of isolates were in line with this current study results.

Molecular studies done by multiplex PCR, revealed that the most prevalent genes were bla_{NDM} 44% (97/217), bla_{OXA}-*48* 39% (86/217) and bla_{KPC} 9% (20/217) while bla_{VIM} and bla_{IMP} genes were not detected, similar results were obtained by Haji et al. [23] where bla_{NDM} was the most frequent gene (85%) among the Enterobacteriaceae isolates followed by bla_{OXA-48} (75%), while bla_{KPC} gene was scarcely detected and comes with the percentage of (3.6%).

On species level, the findings from a study conducted on *Klebsiella pneumonia* samples collected from patients at two Egyptian university hospitals [24] indicate a significant similarity with our own research findings concerning the prevalence of bla_{NDM}. In that study, NDM genes were identified in 56.5% of the isolates. Furthermore, in line with the findings of our study, a separate research conducted at Assiut University Hospital also revealed the bla_{NDM} gene as the most prevalent gene in *Enterobacter cloacae* isolates obtained from from pediatric patients[25].

The present study results are reinforced by another study [26] which revealed that out of 37 carbapenemase-harboring *Klebsiella pneumoniae* isolates 24 (64%) isolates harbored both bla_{NDM} and bla_{OXA-48} genes, while only 11 (29%) isolates carried bla_{NDM-1} gene.

CONCLUSION

Taken together, this study highlights the high prevalence and complex antibiotic resistance patterns of MDR bacteria in NICUs and PICUs. The findings underscore and emphasize the immediate need for robust infection control measures, prudent antibiotic prescription practices, and comprehensive antibiotic stewardship programs in NICUs and PICUs to mitigate the emergence and spread of MDR bacteria. Further research is warranted to understand the local epidemiological factors and the underlying mechanisms of antibiotic resistance in order to inform evidence based strategies for managing MDR bacteria in pediatric critical care settings.

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