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ORIGINAL ARTICLE

Phenotypic and Genotypic Characterization of Carbapenem – Resistant Gram – Negative Bacteria among Pediatrics with Positive Blood Cultures

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ABSTRACT

Key words:
Carbapenemases, lateral flow immunoassay, Conventional PCR, OXA-48

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Background: *Carbapenems are β-lactam antibiotics, which are regarded as one of the final options to treat severe infections resulting from multidrug-resistant organisms. Carbapenem resistance is mostly caused by carbapenemases, which are enzymes that break down this kind of β-lactam antibiotic. It can also occur through non-enzymatic processes such as efflux or changes in porin structure when AmpC or extended-spectrum β-lactamases (ESBL) are present. Carbapenemase-mediated resistance poses a far greater threat.* ***Objectives:*** *The aim of the study was to detect the underlying mechanisms of resistance of carbapenem resistant gram negative bacteria by phenotypic detection (lateral flow immunoassay) directly from positive blood cultures& genotypic detection (Conventional PCR).* ***Methodology:*** *This research was performed on clinical isolates of 25 positive blood cultures of carbapenem resistant Gram -ve bacteria from pediatric cases at Beni-Suef University Hospital. The isolates underwent identification & antimicrobial susceptibility testing by means of VITEK 2 compact system. We assessed a rapid lateral flow immunoassay (LFIA) directly on positive blood culture bottles for carbapenemase genes (OXA-48, KPC, NDM, VIM, and IMP) & compared outcomes with those obtained utilizing conventional polymerase chain reaction (PCR) techniques.* ***Results:*** *PCR test presented that OXA-48 was the most predominant genotype (96%), and the least prevalent was IMP (56%). We evaluated the results of the lateral flow immunoassay and found that the sensitivity of each type was in the range of 71.4%–100% and the specificity of each type was from 0%–90.9% in comparison with PCR as the gold standard.* ***Conclusion****: The lateral flow immunoassay is an effective & efficient phenotypic test for quickly identifying carbapenemase-generating isolates straight from blood culture bottles. It may also be utilized as a point-of-care test.*

INTRODUCTION

Carbapenems belong to β-lactam group of antibiotics and have traditionally been regarded as the final option for treating Gram-ve infections that are resistant to several antibiotics [1].

Carbapenemases synthesis is the primary factor contributing to carbapenem resistance in Carbapenem-resistant Enterobacterales (CRE) strains [2].

Carbapenemases are usually classified into 3 categories of β-lactamases: classes A, B, & D. *Klebsiella pneumoniae* carbapenemases *(KPCs)* are the prevailing class A carbapenemases. *NDM, IMP, & VIM* are classified as class B metallo-β-lactamases & serve as representatives of this class. Oxacillinase, also known as *OXA-48*-like β-lactamases, is a predominant kind of Class D carbapenemase [3].

Global health risks are posed by the spread of Enterobacterales that produce carbapenemase [4]. Because carbapenemases can break down all b-lactam antibiotics, only a few drugs may effectively treat CPE-induced infections. Consequently, these illnesses are often associated with high death rates and worse prognosis[5].

Treating infections caused by CRE strains is sometimes challenging. ceftazidime/avibactam (CZA) is a new combination of beta-lactam and beta-lactamase inhibitors that has a strong inhibitory impact on class A, C and certain D β-lactamases (as *OXA-48*-like). However, it is not effective against class B metallo-β-lactamases [6].

If enterobacteriaceae isolates develop *NDMs* (or any other metallo-β-lactamase), the recommended antibiotic treatment is a combination of CZA & aztreonam[7]. Hence, the prompt and dependable differentiation of these carbapenemases would offer crucial insights for suitable therapy [8].

A novel immunochromatographic detection test called **Carbapenem-Resistant K.N.I.V.O. Detection K-Set (Goldstream, Beijing Gold Mountainriver Tech Development Co., China)**. This lateral flow test is designed to detect the five primary forms of carbapenemases, specifically *KPC-type, NDM-type,IMP-type,VIM-type,&OXA-48*-type carbapenemases, from both bacterial culture & blood culture bottle samples. The molecular identification of genes producing carbapenemase is a compelling option. However, it is expensive, time-consuming, and not easily feasible on a global scale [9]. Our study aimed to assess phenotypically and genotypically carbapenem resistant gram negative bacilli.

METHODOLOGY

***Isolation and Identification of bacteria:**

From June 2022 to January 2023, samples sent to the microbiology laboratory of Beni-Suef University Hospital yielded clinical isolates of 25 positive blood cultures of carbapenem-resistant Gram-ve bacteria.

The investigation received an approval from Beni-Suef University's Faculty of Medicine Research Ethical Committee (**Approval No. FMBSUREC/ 08052022/ Abdelwahed).**

Gram-negative isolates were obtained after culture of blood specimens on Bactec blood culture bottles and sometimes manual blood culture bottles, then subcultured on blood, chocolate and MacConkey agar ***(Oxoid Co., England),*** then all were incubated aerobically at 37°C for 24 hours[10]**.** An automated antimicrobial susceptibility system **(VITEK 2 COMPACT)** identified the isolates.

***Detection of carbapenem-resistant Gram negative bacteria:**

If gram negative bacteria can either not react to at least single carbapenem antibiotic (ertapenem, meropenem, or imipenem) or if they produce carbapenemase enzyme that makes bacteria resistant to carbapenem antibiotics, they are considered carbapenem resistant [11].

Antimicrobial susceptibility was done for isolated gram negative bacteria by an automated antimicrobial susceptibility system (***VITEK 2 COMPACT***). During the study period, all strains of CR Gram-negative isolates were preserved on glycerol broth in two epindorph tubes and stored at -70 C for subsequent PCR analysis.

*** Identification of carbapenemease-producing isolates directly from blood culture bottles using lateral flow immunoassay:**

(The Goldstream® Carbapenem-resistant K.N.I.V.O. Detection K-Set, China) kit, which is a sandwich immune chromatographic assay, was used for 25 positive blood culture bottles**.**

Detection Procedure:

We conducted the procedure directly on the blood sample that tested positive from the vials. An aliquot of 1.8 ml was obtained and subjected to centrifugation at 14,000 rpm for 90 s. Following the liquid removal, we mixed the solid residue with 1 ml of DW for 1 minute to induce hemolysis, and then centrifuged using the same parameters [12]**.**

We added 5 drops of the sample treatment solution into a disposable microcentrifuge tube, then dipped and inserted a disposable inoculation loop into the container to add the supernatant. We mixed well. The cassettes A and B were placed horizontally, and fifty µL of mixture was added to the sample hole, respectively. We waited 10–15 minutes, then read and recorded the results.

Interpretation:

- The presence of single or more red lines in test area, regardless of line intensity, indicated a positive finding for its corresponding gene type.
- A single control line (C line) indicated a negative finding .
- If control line was not visible, the outcome was deemed invalid, and the test was repeated.

*** Molecular identification of *OXA 48, NDM, KPC, VIM, & IMP* genes:**

A PCR procedure was done for 25 carbapenem-resistant Gram-ve isolates in the Microbiology Department for Research & Postgraduate Studies in Faculty of Pharmacy, Beni-Suef University**.**

Extraction of DNA:

Extraction of DNA followed the protocol outlined in reference [13].

DNA amplification*:*

*** Willowfort, UK,** supplied the PCR master mix **(COSMO PCR RED MASTER MIX (2X.)**

* Primers: **Willowfort, UK**, synthesised them, as indicated in the following table:

Table 1: Gene primers for *KPC*,*OXA-48*,*VIM*, *IMP* [14], *NDM*[15]. Bp=base pair

Gene	Primer	Reverse primer	Molecular size
KPC	CATTCAAGGGCTTTCTTGCTGC	ACGACGGCATAGTCATTTGC	498 bp
OXA-48	GCTTGATCGCCCTCGATT	GATTTGCTCCGTGGCCGAAA	238 bp
NDM	GGTTTGGCGATCTGGTTTTC	CGGAATGGCTCATCACGATC	521 bp
VIM	GGTGTTTGGTCGCATATCGCAA	ATTCAGCCAGATCGGCATCGG	502 bp
IMP	TCGTTTGAAGAAGTTAACG	ATGTAAGTTTCAAGAGTGATGC	568 bp

PCR thermal cycling conditions:

DNA thermal cycler **(Biometra Analytic Jena Company, Germany & Sensquest labcycler, Germany.)** were utilized. Thermal cycling conditions were summed up in table 2

Table 2: PCR conditions for *KPC*,*OXA-48*,*VIM*, *IMP* [14], *NDM*[15].

	OXA-48	*KPC*	*NDM*	*VIM*	*IMP*
initial denaturation	95°C for 5 min	95°C for 5 min	95°C for 5 min	95°C for 5 min	95°C for 5 min
Denaturation					94°C for 45 sec
Annealing	54 °C for 45 sec	54 °C for 45 sec	54 °C for 45 sec	58 °C for 45 sec	46.5 °C for 45 sec
Extension	72°C for 1 min	72°C for 1 min	72°C for 1 min	72°C for 1 min	72°C for 1 min
previous steps were Repeated for thirty five cycles					
final extension	72°C for 10 min	72°C for 10 min	72°C for 10 min	72°C for 10 min	72°C for 10 min

Detection of PCR products:

Using a UV transilluminator and gel electrophoresis, amplified genes were detected. **(Whatman, Biometra, Germany)**

Statistical Analysis

We used Statistical Package for Social Science (SPSS) version 22 for Windows 7 (SPSS Inc., Chicago, IL, USA) to analyze the data. Simple descriptive analysis of quantitative parametric data expressed as percentages & numbers for qualitative data & arithmetic means for calculating central tendency & SD for dispersion, respectively.

For qualitative data

- **Chi-square** test was utilized to compare among 2 of > 2 qualitative groups.
- **MC-Nemar** test was utilized for paired dependent qualitative data.
- **Sensitivity and specificity test for testing new test with ROC curve** "Receiver Operating Characteristic".
- A **P-value below 0.05** was considered as statistically significant

RESULTS

The 25 positive blood cultures of carbapenem-resistant Gram negative bacteria were examined directly **by The Goldstream® Carbapenem-resistant K.N.I.V.O. Detection K-Set,** and then carbapenem-resistant Gram-negative isolates were examined by **PCR** to be compared by results of lateral flow immunoassay.

The PCR test showed that 96% of cases were +ve for the *OXA-48* gene, followed by 86% of cases were +ve for VIM gene, 80% were +ve for *NDM* gene, 64% were +ve for *IMP* gene, finally 56% were +ve for the *KPC* gene, as shown in Table 3

Table 3: Frequency of five genes by PCR among study group.

Genes	Pcr results (Variables n=25)	
	Negative	**Positive**
***Oxa-48* gene**	1(4%)	24(96%)
***NDM* gene**	5(20%)	20(80%)
***KPC* gene**	11(44%)	14(56%)
***VIM* gene**	8(32%)	17(86%)
***IMP* gene**	9(36%)	16(64%)

No statistically significant variance was noted in identification of positive *OXA-48, NDM, KPC, VIM, & IMP* genes with p-values above 0.05 between PCR and lateral flow tests as shown in table 4**.**

Table 4: Comparisons of the five genes PCR results and lateral flow results among study group.

***Oxa-48* gene**	**PCR results**				**P-value**	**Sig.**
	Negative (N=1) (4%)		**Positive** (N=24) (96%)			
	No.	**%**	**No.**	**%**		
Lateral flow						
Negative	0	0%	4	16.7%	0.37	NS
Positive	1	100%	20	83.3%		
***NDM* gene**	**PCR results**				**P-value**	**Sig**
	Negative (N=5)		**Positive** (N=20)			
	No.	**%**	**No.**	**%**		
Lateral flow						
Negative	2	40%	2	10%	0.99	NS
Positive	3	60%	18	90%		
***KPC* gene**	**PCR results**				**P-value**	**Sig.**
	Negative (N=11)		**Positive** (N=14)			
	No.	**%**	**No.**	**%**		
Lateral flow						
Negative	10	90.9%	4	28.6%	0.37	NS
Positive	1	9.1%	10	71.4%		
***VIM* gene**	**PCR results**				**P-value**	**Sig.**
	Negative (N=8)		**Positive** (N=17)			
	No.	**%**	**No.**	**%**		
Lateral flow						
Negative	5	62.5%	2	11.8%	0.99	NS
Positive	3	37.5%	15	88.2%		
***IMP* gene**	**PCR results**				**P-value**	**Sig.**
	Negative (N=9)		**Positive** (N=16)			
	No.	**%**	**No.**	**%**		
Lateral flow						
Negative	5	55.6%	0	0%	0.13	NS
Positive	4	44.4%	16	100%		

A statistically significant sensitivity to lateral flow test in the diagnosis of the *KPC* gene with a sensitivity of 71.4 & specificity of 90.9 percent with a p-value of 0.009. Furthermore, lateral flow tests showed a statistically significant sensitivity in the diagnosis of *VIM* and *IMP* genes with a with a sensitivity of 88.2% and 100% and a specificity of 62.5% and 55.6%) with p-values of 0.04 & 0.02, correspondingly, but *OXA-48* and *NDM* genes showed no statistically significant results, as sensitivity was 83.3% and 90% and specificity was 0% and 41.7% with p-values of 0.78 & 0.31, correspondingly, as shown in table 5.

Table 5: Sensitivity and specificity of different genes in comparison to PCR among study group at baseline time:

Variable	Sensitivity	Specificity	AUC	p-value (95% CI)
***OXA-48* gene**	83.3%	0%	41.7%	0.78
***NDM* gene**	90%	40%	65%	0.31
***KPC* gene**	**71.4%**	**90.9%**	**81.2%**	**0.009***
***VIM* gene**	**88.2%**	**62.5%**	**75.4%**	**0.04***
***IMP* gene**	**100%**	**55.6%**	**77.8%**	**0.02***

DISCUSSION

Carbapenemases are grouped into three β-lactamase Ambler classes: class A, that has *KPC* as its most common type; class B, which has metallo β-lactamases (MBLs) like *NDM, VIM, & IMP*; and class D, which has *OXA-48*-like[16]. Quickly detecting & identifying carbapenemases can aid in preventing the transmission & infection control of carbapenemase-producing isolates within healthcare facilities[17,18]. Recently, there has been significant progress in rapid diagnostic testing using LFAs (lateral flow immunoassays) to detect enzymes linked to antimicrobial resistance. These LFAs have shown similar performance to the gold standard polymerase chain reaction-based techniques & can be conveniently used in clinical microbiology labs without the need for specialized staff or excessive expenses [19].

As the PCR is the gold standard , we detected that *OXA-48* was the most common gene detected among CRE by a percentage of 96%, which was similar to several studies in Egypt [20] that reported 72.5% of CRE isolates from cases in Al-Zahraa & Helwan University hospitals in Cairo, Egypt, harboured the *blaOXA-48* gene. Also, Osama et al[21] stated that *OXA-48* was the most common carbapenamase gene detected by real-time PCR among 149 Gram-negative isolates obtained from paediatric cancer cases in Egypt, but Pingjuan Liu,.et al [8] reported that Klebsiella pneumoniae, which produces *KPC* carbapenemase, was the predominant form of CRE. This suggests that CPE exhibits distinct genetic and epidemiological traits across various geographical areas. Hence, it is crucial to closely observe & acquire expertise in the traits of enzyme synthesis in different strains within each region.

The 2nd common carbapenemase gene was *NDM* (80%); Wassef et al, [22] identified NDM in only twenty four percent of isolates, and Ghaith et al , [23] identified *NDM* with high percentage of 85% in diverse biological specimens collected from cases admitted to Beni-Suef University Hospital.

In our study, for phenotypic detection of carbapenamases genes, we utilised the carbapenem-resistant K.N.I.V.O. detection K-Set (Beijing Gold Mountainriver Tech Development Co., China) to be assessed for 1st time, with sensitivity in range of 71.4 percent to 100 percent & specificity in the range of 0% to 90.9%, respectively.

K-Set is a commercially available set of immunological enzyme detection kits specifically designed to detect five significant kinds of carbapenemases: *KPC, NDM, VIM, IMP, & OXA-48*. Our study indicates that K-Set is very suitable for the identification of the five major carbapenemases, in comparison to the PCR approach, with a sensitivity of 100% for *IMP*, 90% for *NDM*, 83.3% for *OXA*-48, 88.2% for *VIM*, and 71.4 for *KPC* directly from blood culture bottles, which was almost in line with the outcomes of Pingjuan Liu et al.[8] who reported that, compared with the polymerase chain reaction technique, K-Set is very suitable for detecting 5 major carbapenemases, with sensitivity of one hundred percent for *IMP*, 100% for *NDM*, 100% for *OXA-48*, 100% for *KPC*, and 100% for *VIM*. Aurélie Cointe et al., [24] revealed that 100% of *OXA-48*-like isolates (30/30) & *KPC*-type isolates (17/17) were accurately identified by RESIST-4 O.K.N.V. test when applied directly to positive blood vials.

Several investigations have assessed the efficacy of NG-test Carba 5, a type of lateral flow immunoassay, and have consistently shown high performance on bacterial colonies and positive blood cultures. The overall specificity & sensitivity of this test varied from 97.3 percent to 100 percent & 95.3 percent to 100 percent, correspondingly, as reported in previous studies [25,26,27]. The RESIST-5 O.O.K.N.V. identified the presence of *KPC*-type & *OXA-48*-like carbapenemases in blood cultures with sensitivity of 100 percent for both. However, the sensitivity for detecting *NDM*-type & *VIM*-type carbapenemases was 50.0% & 52.2%, respectively [28].

CONCLUSION

The percentage of carbapenem-resistant Gram –ve bacteria amongst pediatrics is increasing in hospitals in our country due to inappropriate infection control measures. The *OXA-48, NDM, KPC, VIM, & IMP* are present amongst Gram -ve bacteria in our Beni-Suef University Hospital in different percentages. The lateral flow immunoassay is a good rapid phenotypic test for identifying carbapenamase-producing isolates directly from blood cultures, and it is in good agreement with PCR for identifying the most prevalent carbapenamase genes.

Abbreviations:

CPE: Carbapenemase-producing Enterobacterales
CR: Carbapenem resistant
CRE: Carbapenem resistance Enterobacterales
CZA: Ceftazidime/avibactam
DW: Distelled water
ESBL: Extended-spectrum β-lactamases
KPCs: Klebsiella pneumoniae carbapenemases
LFIA: Lateral flow immunoassay
PCR: Polymerase chain reaction

Declarations:

Consent for publication: Not applicable
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Ethical approval: This research was approved by the Research Ethical Committee, Faculty of Medicine Beni-Suef University; **Approval No: FMBSUREC/08052022/Abdelwahed.**

Authors' contributions:
M.E.A., N.M.K., E.M.F., A. A. G., M.S. shared in the laboratory study and data collection, and A. M.S. S. shared the clinical data of the children. All authors shared the study design, draft writing, revising, and approval of the final manuscript.

REFERENCES

1. HandalR, Qunibi L., Sahouri I. et al. "Characterization of carbapenem-resistant acinetobacter baumannii strains isolated from hospitalized patients in Palestine," The Internet Journal of Microbiology. 2017, doi: 10.1155/2017/8012104.
2. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin. Microbiol. Infect Dis.2014;20:821–830 doi: 10.1111/1469-0691.12719.
3. Han R., Shi Q, Wu S, Yin D, Peng M, Dong D et al . Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) Among Carbapenem-Resistant Enterobacteriaceae Isolated From Adult and Children Patients in China. Front. Cell. Infect. Microbiol. 2020;10:314. doi: 10.3389/fcimb.2020.00314.
4. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol. Med. 2012.18,263-272.doi:10.1016/j.molmed.2012.03.003 .
5. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg. Infect. Dis.2014 .20, 1170–1175. doi: 10.3201/eid2007.121004 .
6. Shirley M. Ceftazidime-Avibactam: A Review in the Treatment of Serious Gram-Negative Bacterial Infections. Drugs. 2018;78:675–692.doi: 10.1007/s40265-018-0902x.
7. Falcone M, Daikos GL, Tiseo G, Bassoulis D et al. Efficacy of Ceftazidime-Avibactam Plus Aztreonam in Patients With Bloodstream Infections Caused by Metallo-β-Lactamase-Producing Enterobacterales. Clin. Infect. Dis.2021. Off. Publ. Infect. Dis. Soc. Am., 72, 1871–1878, doi:10.1093/cid/ciaa586.
8. Pingjuan Liu, Jiankai, Deng Siting ,Yi2 Mengmin , Han HP,et al Comparison of the performance of two rapid immunochromatographic methods for the detection of carbapenemase genes among carbapenem-resistant enterobacterales clinical strains: Europe PMC, 2023. doi.org/10.21203/rs.3.rs-2454925/v1 .
9. Sadek M, Demord A, Poirel L, Nordmann P. Fast and reliable detection of carbapenemase genes in various Gram negatives using a new commercially available fluorescence-based real-time polymerase chain reaction platform. Diagn Microbiol Infect Dis.2020;98(3):115127.DOI: 10.1016/j.diagmicrobio.2020.115127 .
10. Carrol KC and Pfaller MA. Manual of clinical microbiology.2019. 12th edition ASM Press.
11. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. Atlanta, GA: CDC 2019
12. Cointe A, Bonacorsi S, Truong J, Hobson C, et al. Detection of Carbapenemase-Producing Enterobacteriaceae in Positive Blood Culture Using an Immunochromatographic RESIST-4 O.K.N.V. Assay. Antimicrob Agents Chemother. 2018 Nov 26;62(12):e01828-18. doi: 10.1128/AAC.01828-18.
13. Aly AM, Adel A, El-Gendy AO, Essam TM, Aziz RK. Gut microbiome alterations in patients with stage 4 hepatitis C. Gut Pathog.2016, 8, 42. doi: 10.1186/s13099-016-0124-2.
14. Mahmoud NE, Altayb HN, Gurashi RM . Detection of carbapenemresistant genes in Escherichia coli Isolated from drinking water in Khartoum, Sudan. J Environ Public Health,2020. https:// doi. org/ 10. 1155/ 2020/ 25712 93
15. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. BioMed research international, 2014,doi.org/10.1155/2014/303104
16. Wirittamulla Gamage Maheshika Kumudunie, Lakmini Inoka Wijesooriya, et al .Comparison of four low-cost carbapenemase detection tests and a proposal of an algorithm for early detection of carbapenemase-producing Enterobacteriaceae in resource-limited settings .PLOS ONE .2021. https://doi.org/10.1371/journal.pone.0245290.
17. Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, et al .A multiplex lateral flow immunoassay for the rapid identification of NDM- , KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother ,2018. 73:909–915.
18. Takissian J, Bonnin RA, Naas T, Dortet L. NG-Test Carba 5 for Rapid Detection of CarbapenemaseProducing Enterobacterales from Positive Blood Cultures. Antimicrob Agents Chemother.2019 ,https://doi.org/10.1128/AAC.00011-19.

19. Hemwaranon P, Srisrattakarn A, Lulitanond A, et al .Recombinase Polymerase Amplification Combined with Lateral Flow Strip for Rapid Detection of OXA-48-like Carbapenemase Genes in Enterobacterales. Antibiotics, 2022
20. Muhammad DM, Elkholy EESM, Elmadbouly AA, Montasser KA. Detection of carbapenem-resistant enterobacteriaceae isolates harboring OXA-48 gene in a clinical setting: a two-center-based study. The Scientific Journal of Al-Azhar Medical Faculty, 2021. , 5(2), 265.
21. Osama D, El-Mahallawy H, Mansour MT, Hashem A, Attia AS. Molecular characterization of carbapenemase-producing Klebsiella pneumoniae isolated from Egyptian pediatric cancer patients including a strain with a rare gene-combination of β-lactamases. Infection and Drug Resistance, 2021.14, 335.
22. Wassef M, Abdelhaleim M, Ghaith D, El-Mahdy Y.Emerging New Delhi metallo-β-lactamase-1-type-producing gram-negative bacteria isolated from Cairo university pediatric hospital, Cairo, Egypt. J Glob Antimicrob Resist, 2016.7:84–87.
23. Ghaith D, Morsy SA, Sebak M. et al. Phenotypic and genotypic characterization of carbapenem-resistant Gram-negative organisms, Beni-Suef, Egypt. Beni-Suef Univ J Basic Appl Sci 2023.12, 61. https://doi.org/10.1186/s43088-023-00400-w.
24. Aurélie Cointe, Stéphane Bonacorsi, Jeanne Truong, Claire Hobson, et al.Detection of Carbapenemase-Producing Enterobacteriaceae in Positive Blood Culture Using an Immunochromatographic RESIST-4 O.K.N.V. Assay. Antimicrob Agents Chemother. 2018 Dec; 62(12): doi: 10.1128/AAC.01828-18.
25. Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, et al .A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother ,2018. 73:909–915.
26. Bodendoerfer E, Keller PM, Mancini S. Rapid identification of NDM-, KPC-, IMP-, VIM- and OXA48-like carbapenemase-producing Enterobacteriales from blood cultures by a multiplex lateral flow immunoassay. J. Antimicrob.Chemother.2019, 74,1749–1751.
27. Giordano L, Fiori B, D'Inzeo T, Parisi G et al. Simplified testing method for direct detection of carbapenemase-producing organisms from positive blood cultures using the NG-test Carba 5 assay. *Antimicrob. Agents Chemother.* 2019, *63*, e00550-19.
28. Bianco G, Boattini M, van Asten S, Iannaccone M, Zanotto E, Zaccaria T, et al. RESIST-5 O.O.K.N.V. and NG-Test Carba 5 assays for the rapid detection of carbapenemase-producing Enterobacterales from positive blood cultures: a comparative study. *J. Hosp. Infect.* 2020.105 162–166.