World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Molecular detection of carbanemase in gram negative bacteria Isolated from intensive care unit patients in wasit province, Iraq

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World Journal of Biology Pharmacy and Health Sciences, 2022, 12(02), 130–139

Publication history: Received on 28 September 2022; revised on 12 Novemebr 2022; accepted on 15 November 2022

Article DOI: https://doi.org/10.30574/wjbphs.2022.12.2.0177

Abstract

ICUs are crucial medical facilities for treating life-threatening diseases, and bacterial contamination of ICU equipment and devices is a significant cause of nosocomial infections. In this study, a total of 100 clinical specimens (urine, sputum and pus) were collected from patients admitted in the ICU. Results showed eighty two were positive growth culture. 30(36.5%) were female and 52(63.5%) were male patients admitted to the intensive care unit distributed 69 (84.1%) gram-negative bacteria .while gram positive 13(15.8%), as well as 20 pure isolates of some places, materials and equipment distributed 12 (75%) Gram negative bacteria .While 8 (25%) it is gram positive bacteria. Results of antibiotic susceptibility test only Trimethoprime was effective choice against *Acinetobacter baumannii*. Phenotypically survey of carbapenem *klebsiella pneumoniae* were 9(47%), *pseudomonas aeruginosa* was 7(35% of MBL and Carbapenemase and the result of MBL and Carbapenemase for E. Coli 7(43%) similar results (40%). Whereas, Genotypically the most prevalent gene in E.Coli 1 (10%), the result was for *Burkholderia cepacia* bla_{VIM} 1(100%), its prevalence was 7 (53%) among bacteria *Klebsiella pneumonia* for NDM, the result of bacteria *Pseudomonas aeruginosa* for this gene was 4(33.3%) and Escherichia Coli was 1(10%), its prevalence was (100%) among klebsiella oxytoca for KPC.

Keywords: Intensive care unit; Bacteria characterization; Antibiotic susceptibility Phenotypes and genotype of MBL; Carbapenemase gram negative bacteria

1. Introduction

ICUs are crucial medical facilities for treating life-threatening diseases, and bacterial contamination of ICU equipment and devices is a significant cause of nosocomial infections [1]. More than one million newborn fatalities are recorded globally each year due to nosocomial infections caused by bacterial contamination of ICU equipment and devices [2]. Additionally, fungus and viruses pollute and persist for varying lengths on the surfaces, tools, and interior environment of the NICU [3]. Generally bacteria are the most predominant cause, then fungi and viruses, however it also depending on the healthcare facility location, the environment of healthcare, and the population of patient [4].

Although many seriously ill people get resistant bacterial strains ultimately, the UTs alone represent up to 35–40% of nosocomial infections. Typically, Gram–negative organisms are the common cause of these infections which are related to indwelling catheters use or urinary obstruction. The second most common cause is wound infections, represent for up to 25–30% of infections in intensive care units. 5–10% of healthcare-associated infections (HAIs) are a significant burden on the global public health system since they are one of the main causes of morbidity and death among hospitalized patients [6, 7]. The risk of HAI is five to seven times higher for patients in the intensive care unit (ICU), and ICU infections account for 20% to 25% of all HAI in a hospital. Immunosuppressive medications, more invasive device usage, and the inappropriate use of antibiotic treatment in ICUs are all significant factors [8].

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Production of -lactamases, mutations that change the expression and/or activities of efflux pumps, porins, and penicillin-binding proteins (PBP), and carbapenem-hydrolyzing -lactamases (Carbapenemases) are the main tolerance mechanisms against carbapenems [9]. The aim was presented to detect spread of bacterial isolates from patients in ICU in Kut City, Wasit Province, Iraq, and to characterize it at the level of molecular analyses of its Phylogenic and antimicrobial resistance genes.

2. Material and methods

A cross-sectional study was done in the intensive care unit (ICU) of the Alzahraa, Alkarama hospitals from the 3rd October 2021 to 20th February 2022. A total of 100 clinical samples, including: urine, sputum and pus culture media such as mannitol salt agar, MacConkey agar, blood agar, and chocolate agar. The growth showed different bacterial colonies whose morphological and biochemical characteristics were tested. Then DNA was extracted; purity and concentration were confirmed with Nanodrop. The purity of gram-negative bacteria (1.8-2), and the concentration was between 50-360 ng/µl.

Table 1 Primers' sequence of gram-negative bacteria

Multiplex PCR pool	Primers	Sequences (5'-3')	Size(bp)	References
Multiplex-V: IMP, VIM and SIM	MultiCaseIMP-F	GGAATAGAGTGGCTTAATCTC	180	(Ugwu *et al.*, 2020)
	MultiCaseIMP-R	CCAAACYACTASGTTATCT		
	MultiCaseVIM-F	GATGGTGTTTGGTCGCAT	390	
	MultiCaseVIM-R	CGAATGCGCAGCACCAGA		
	MultiCaseSIM-F	TACAAGGGATTCGGCATCG	570	
	MultiCaseSIM-R	TAATGGCCTGTTCCCATGTG		
Multiplex VI: GIM and SPM	MultiCaseGIM-F	TCGACACACCTTGGTCTGAA	477	
	MultiCaseGIM-R	AAC TTCCAACTTTGCCATGC		
	MultiCaseSPM-F	AAA ATCTGG GTA CGC AAA CG	271	
	MultiCaseSPM-R	ACATTATCCGCTGGAACAGG		
Monoplex NDM-1	NDM-1-F	ACCGCCTGGACCGATGACCA	264	
	NDM-1-R	GCCAAAGTTGGGCGCGGTTG		
Monoplex KPC	KPC-F	CATTCAAGGGCTTTCTTGCTGC	538	
	KPC-R	ACGACGGCATAGTCATTTGC		

2.1. Phenotypic Detection of Carbapenemases Production

The double disk synergy approach was used to identify metallo -lactamases (MBLs), as stated by [10]. The turbidity of a bacterial isolate suspension in peptone water was measured and found to be 0.5 McFarland, thus the agar plates used for inoculation were prepared according to these standards and incubated at 37 ℃ for 2 hours. Both imipenem and meropenem disks (10 µg) and their respective EDTA controls (15 mm) were incubated at 37 °C for 18 hours. Carbapenem non-susceptible isolate showing an increase of approximately ≥ 5 mm in the inhibition zone with carbapenem compared to carbapenem-EDTA disk alone was considered as a producer of MBL

Table 2 Thermal cycling program for multiplex pool.3 MBL group

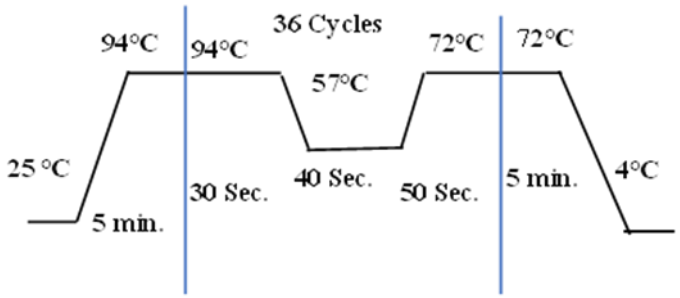
Genes	PCR cycling profile	Products size
bla*$_{VIM,}$ *IMP,GIM, SPM,SIM		390bp 180bp 477bp 271bp 570bp

Table 3 Thermal cycling program for monoplex NDM

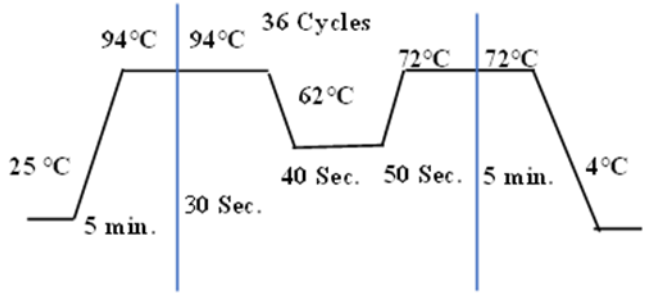
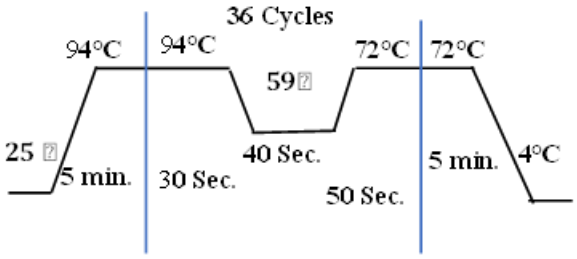
Genes	PCR cycling profile	Products size
***bla*$_{NDM-1}$**		264bp

Table 4 Thermal cycling program for monoplex KPC

Genes	PCR cycling profile	Products size
***bla*$_{KPC}$**		538bp

According to [11,12] who adapted this protocol as a reliable and efficient tool for the identification via multiplex PCR assays of the most common encoding of the beta-lactamase genes for gram negative bacteria ,thereafter gel electrophoresis and Documentation [13] and finally the data's statistical analysis has been carried out with the use of SAS (Statistical Analysis System - version 9.1) [14]. Furthermore, the percentages have been compared viathe use of Chi-square test. $P < 0.05$ is considered statistically significant.

3. Results and discussion

The current study was conducted on a total one hundred clinical specimens (urine, sputum and pus) were collected from patients admitted in the ICU. Eighty two were positive growth culture distributed according to the patient's age, the highest incidence was among 20-29 age groups with (25.6 %). While the lowest incidence was among (50-59) and (70-79) age group (3.6%), (3.6%) respectively ,the mean ±SD of age was 15.556 ranging from 1 years to 90 years. Eighty two pure positive culture, 30(36.5%) were female of patients 52 (63.5%) were male and admitted to the intensive care unit distributed 69 (84.1%) gram-negative bacteria .while gram positive 13(15.8.

Bacterial isolates that were obtained from the clinical specimens have been initially characterized based on the cultural morphology as well as the biochemical tests. Results of culture showed colonies on MacConkey agar pink color with precipitation of bile salt around colonies, the refers to *Escherichia coli* isolates while results of biochemical tests for *Escherichia coli* were had given positive test for catalase, indole, methyl red, but negative for oxidase, voges-proskauer, simmon citrate, and TSI test showed A/A with gas , without H2S. This results diagnostic for *Escherichia coli* .Similar results was recorded by [15–17].

Results of identification of *klebsiella pneumoniae* were showed pink lactose fermenter mucoid colonies on MacConkey agar. Positive test for urease, voges-proskauer, simmon citrate , but negative for oxidase, methyl red, motility test, indole and TSI test showed A/A with gas , without H_2S . Further confirmation done using Vitek2. Similar results were recorded by [18, 19].

Results of *Acinetobacter baumannii* showed nonmotile and give negative result to oxidase, indole, methel red, voges proskauer and avirable to urea. All isolates were positive to catalase , simmons citrate and triple-sugur-iron test was alkaline / no change, these results are identical with those obtained by [20, 21]. The results of *Pseudomonas aeruginosa* showed that all isolates had given negative result for indole, methyl red, voges proskauer and urease test also, citrate assimilation was positive, motility was positive and TSI alkaline / alkaline or Alkaline / no change with no production of H_2S and gas and isolated from cetrimide agar colonies appeared mucoid, smooth in shape, fruity odour, fluorescent green, and creamy pigments these results agreed with what mentioned by [16, 21–23].

The results of *Proteus mirabilis* appear as bacilli, rapidly motile by flagella. Swarming phenomena on blood agar plate. It is non-lactose fermenter so it gives a pale colony on macConky agar. Negative test for indole and voges-proskauer but positive test for urease, simmon, s citrate, H_2S is produced, nitrate reduction motility and methyl reed .Similar finding was recorded by [18, 21].

The results of *Serratia. marcescens*, showed that isolate had given result for indole , urease test , citrate assimilation , motility and catalase positive . Methyl red, voges proskauer and oxidase negative and TSI alkaline / acidic or acidic / acidic with no production of H_2S and gas.The results of *Burkholderia* cepacia complex showed that isolate had given a result at negative for indole .But positive for methyl red, voges prosumer, urease test, citrate assimilation, motility,catalase and oxidase ,TSI alkaline / acidic with no production of H_2S and gas.

The results of *Pantoea spp* showed that isolate had given result for indole methyl red, urease test, motility, and oxidase negative . Voges-proskauer citrate assimilation, catalase positive, TSI alkaline / acidic with no production of H_2S and gas. The results *Enerobacter cloacae* indicated that isolate had given result for indole, methyl red,urease test , motility and oxidase negative. Voges-proskauer, citrate assimilation and catalase was positive. TSI alkaline / acidic with no production of H_2S and gas. The results of *staphylococcus aureus* appeard that isolates had given result for indole was negative , methyl red was positive , voges-proskauer was positive , urease test was positive , citrate assimilation was positive, motility was negative and TSI acidic / acidic with no production of H_2S and gas , catalase was positive , coagulase was positive and oxidase was positive this results are consistent with findings from other Iraqi studies [23,24]. Also similar result was recorded by [25–27]. On blood agar *staphylococcus haemolyticus* fermenters as yellow colonies. Further biochemical tests were necessary for identification of *staphylococcus haemolyticus* from other species, all isolates were positive to catalase test, but negative for coagulase and oxidase [28]. The results of *Enterococcus faeciu* showed that isolate had given negative results for indole, urease test, citrate assimilation, motility, catalase and oxidase. But positive result for voges-proskauer.

3.1. Antibiotic susceptibility test of *Acinetobacter baumannii*

The maximum resistance level of this study to (Ampicillin, Piperacillin Cefotaxime , Ceftriaxone , Ceftazidime , Cefixime , Cefepime , Cefoxitin , Nitrofurantion , Piperacillin -tazobactam , Amoxicillin -clavulanic acid Amikacin , Gentamicin,

Nalidixic acid , Imipenem , Meropenem ,Levofloxacin and Ciprofloxacin (100%) respectively, however the intermediate resistance level of study (Trimethoprime 50%).

In this study, the results were as shown that *Acinetobacter baumannii* is highly resistant, and these results were the same as with [19]; These results were comparable with results of studies conducted by [29] in North eastern Ethiopia.

3.2. Phenotypic detection of carbapenem producers

By the screening for MBL and Carbapenemase for the *klebsiella pneumoniae* were 9(47%) , similar results (42%) It was reported in another study [30]. The result of MBL and Carbapenemase for *klebsiella oxytoca* was 1(100%) compatible with the result [31] phenotype testing for carbapenemase production showed that the carbapenem-resistant isolates were positive MBL .Our study were results for *pseudomonas aeruginosa* was 7(35%) of MBL and Carbapenemase , similar results studies for MBL and Carbapenemase by [32] and [33] with (26.5%) and (37%) respectively .The result of MBL and Carbapenemase for *E. Coli* 7(43%) similar results (40%) .It was reported in another study [34]. By the screening for MBL and Carbapenemase for the *Proteus mirabilis* were 2(40%) similar results (50%) It was reported in another study [35]. In the our studies the result of MBL and Carbapenemase for *Acinetobacter baumannii* 1(50%) are similar with the study performed by [36] (60%) .Carbapenemase enzymes confer resistance to broad-spectrum β-lactam antibiotic and it is a one of the important carbapenem resistant mechanisms in gram-negative bacteria.

3.3. Carbapenemase ESBLs

In this study to investigate the molecular mechanisms of carbapenem resistance in Gram negative bacteria include most common MBLs enzymes are belonged to *VIM, IMP, SPM, GIM, SIM , NDM* and *KPC* families [37].

3.4. IMP, VIM, SIM

In the present study, no PCR-amplification products with *bla$_{IMP}$, bla $_{VIM}$, and bla $_{SIM}$.* Genes among Pseudomonas *aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Proteus mirabilis* and *Klebsiella oxytoca* isolates. In the present study bla$_{IMP}$ was revealed that the most prevalent gene in (10%) among 1 *Escherichia Coli* from (10) by phenotypic and confirmatory test, these results in close with [38–40] reported the prevalence of gene *bla$_{IMP}$* (3%); (30%) ; (12%) respectively. It also agrees with a study in Wasit (Nasser et al., 2022) found the prevalence of gene bla$_{IMP}$ (22%). While the result was for *bla$_{VIM}$* (100%) for 1 *Burkholderia cepacia* from (1), interestingly, these findings are in consistent with a previous study reflected a high rate of ESBL genes in clinical isolates [41, 42] who found that its isolates she carries this gene bla$_{VIM}$ (78%) ; (100%) respectively. No PCR-amplification products with SIM gene in any isolates of gram negative bacteria in this study. The agarose gel of PCR products was shown in Figure (1), (2).

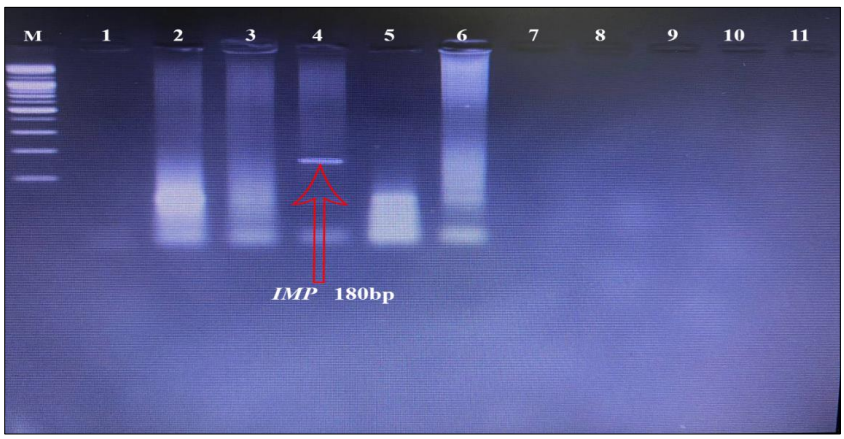


Figure 1 Agarose gel electrophoresis of image that showed PCR product analysis for *IMP, VIM, SIM* gene from gram negative bacteria. M (Marker ladder 100-2000bp). Lane (1-11): some *IMP* gene *Escherichia Coli* isolate at (180 bp) gene product size

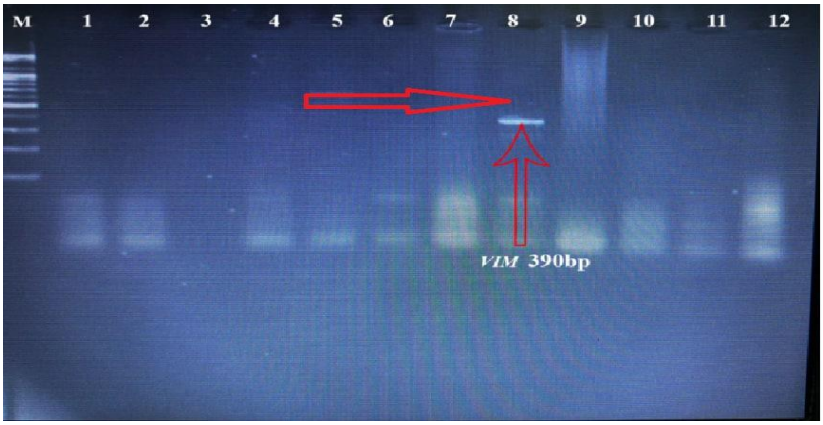


Figure 2 Agarose gel electrophoresis of image that showed PCR product analysis for *IMP, VIM, and SIM* gene from gram negative bacteria. M (Marker ladder 100-2000bp). Lane (1-12): some *VIM* gene *Burkholderia cepacia* isolate at (390 bp) gene product size

3.5. GIM, SPM

No PCR-amplification products with *SPM* and *GIM* genes in any isolates of gram negative bacteria in this study.

3.6. NDM

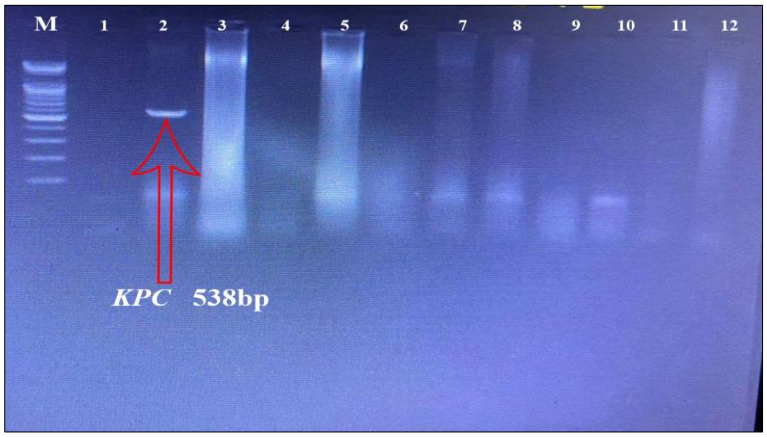
In this study, No PCR-amplification products with *bla* $_{NDM}$ among *Acinetobacter baumannii*, *Proteus mirabilis* and *Klebsiella oxytoca*) . Regarding to *NDM* gene in the present study, its prevalence was *7* (53%) among bacteria *Klebsiella pneumoniae* The results of our current study are consistent with the results of these studies in BraziL [43,44] in Sergipe, Brazil , It was agreed with these studies because its isolates they have this gene *NDM* (59.2%) ; (50.3%) respectively .It does not agree with this study in Wasit [19] because it does not have this gene *NDM*. whereas the result of bacteria *Pseudomonas aeruginosa* for this gene was 4(33.3%) The results of our study are similar to those from previous studies in Diyala, Iraq [45–47] in Iran, It is similar to the result of this study because it contains this gene NDM (25%) ; (4%) ; (35%) respectively. The result of this gene among bacteria *Escherichia coli* was 1(10%),this result agreed with the results in Pakistan [48–50] who observed in these studies that its isolates carry this gene *NDM* (3.5%) ; (12.9%) ; (13.5%) respectively. While it does not agree with this study in Wasit [17] because its results do not contain this gene *NDM*. The agarose gel of PCR products was shown in Figure (3).

Figure 3 Agarose gel electrophoresis of image that showed PCR product analysis for *NDM* gene from gram negative bacteria. M (Marker ladder 100-2000bp). Lane (1-12): some *NDM* gene *Pseudomonas aeruginosa*, *Klebsiella pneumoniae and E.coli* isolates at (264 bp) gene product size. (Agarose con. =2%, voltage =100 volts (40min)

3.7. KPC

In the present work, No PCR-amplification products with bla $_{KPC}$ gene among *Pseudomonas aeruginosa* , *Klebsiella pneumoniae , Proteus mirabilis , Burkholderia cepacia , Acinetobacter baumannii* , and Escherichia coli ,its prevalence was (100%) among klebsiella oxytoca ,this result agrees with another studies in Tehran Hospitals, Iran by [51,52] We

noticed in these studies that isolates carry this gene KPC (99%) ; (96%) respectively. The agarose gel of PCR products was shown in Figure (4).



(agaroe con. =1.5%, voltage =100 volts for (40min).

Figure 4 Picture of the PCR product analysis for the *KPC* gene from gram negative bacteria on an agarose gel electrophoresis. M (Marker ladder 100-2000bp). Lane (1-12): some *KPC* gene *klebsiella oxytoca* isolates at (538bp) gene product size

4. Conclusion

According to the findings of current study in ICU ,male are more frequently admitted and admitted more frequently than females, The highs frequency of specimens collection from intensive care unit patients were isolated from sputum and the gram-negative bacteria were the most common than the positive bacteria. Results of antibiotic susceptibility test only trimethoprime was effective choice against *Acinetobacter baumannii.* Phenotype and genotype for *klebsiella pneumoniae* of carbapenemase results appear that bla_{NDM} the most prevalenrt, while, no isolate have bla_{IMP}, bla_{VIM},bla_{SIM} ,bla_{GIM} ,bla_{SPM} and bla_{KPC} .genes . carbapenemase gene for *pseudomonas aeruginosa* was bla_{NDM} and no isolate have bla_{IMP}, bla_{VIM},bla_{SIM} ,bla_{GIM} ,bla_{SPM} and bla_{KPC} genes. Phenotype and genotype for *Escherichia coli* of carbapenemase genes were bla_{NDM} and bla_{IMP} . Whereas absent bla_{IMP}, bla_{VIM}, bla_{SIM} ,bla_{GIM} ,bla_{SPM} and bla_{KPC} genes in all isolates.

Compliance with ethical standards

Acknowledgments

The authors like to thank the Alzahraa and Alkarama hospitals for their cooperation

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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