

Molecular Study of MDR Bacteria Contaminated Hospital Environment

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Abstract

Some isolates of bacteria were taken from various places inside the hospital, including operating theaters, corridors, ventilation areas, floors, and other samples were taken from patients suffering from antibiotic resistance.

The study aims to discover the multiple resistant bacteria present in the hospital environment, to discover the extent of their resistance, and to detect the gene NDM-1, NDM-2, which when they are present, the bacteria are highly resistant.

When a patient is infected with multiple resistant bacteria, he suffers from all antibiotic resistance and may lead to death, which is the biggest problem in the world today.

Keywords: MDR, NDM-1, NDM-2, Nosocomial Infection.

Introduction

Multi Drug Resistance(MDR) is resistance to more than one antimicrobial. "To date, no standard definition has been agreed upon in the medical community. Multiple definitions are used to describe patterns of multiple drug resistance in Gram-positive and Gram-negative organisms ^{1,2}. The lack of specific definitions of MDR in clinical study protocols creates data that are difficult to compare. The authors and relevant authorities are used to characterize organisms as MDR. And that depends on their sensitivity to the antibiotics applied to the organisms in the laboratory. "Resistant to multiple agents, classes, or subclasses of antimicrobial agents" ³. The most commonly used definition of Gram-positive and Gram-negative bacteria is "it means that any bacteria that are resistant to 3 or more antagonists are An overview of the diversity of

these definitions is provided in a comprehensive review of MDR resistance in *P. aeruginosa* and *A. baumannii* by ⁴. The authors note that a large number of studies do not suggest any specific definitions of MDRs, but most define MDR as "resistance to three or more classes Another method used to characterize bacteria as MDR is when they are "resistant to a major antimicrobial agent" ⁵. These bacterial isolates may have general health significance due to their resistance to only one major antimicrobial agent, but they often exhibit common or shared resistance to multiple classes of antimicrobials, making them Creating an acronym for a bacterium based on its resistance to a major antimicrobial agent (such as methicillin resistance in *S. aureus*, i.e. MRSA) immediately highlights its epidemiological importance ². The advantage of using this approach for monitoring purposes is that it can be applied easily ⁶.

The aim of the study: The present study aimed to determine the prevalence of multi drug resistant bacteria among clinical and environmental samples collected from hospitals in the Najaf province.

Method

Extraction of DNA from Gram-positive, Gram-negative bacteria The DNA extraction method presented

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in this paper is an improved method of the standard phenol/chloroform method⁷.

We eliminated the lysis step that uses SDS/lysozyme or proteinase K, and lysed cells directly by phenol.⁸ To extract the DNA from Gram negative or Gram-positive bacteria, 1 ml cell suspension was centrifuged at 8000g for 2 min, except for *K. pneumoniae* where 13 000g for 10 min was used to pellet cells. After removing the supernatant, the cells were washed with 400 μ l STE Buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0) twice. Then the cells were centrifuged at 8000g for 2 min, except for *K. pneumoniae* (13 000g for 10 min). The pellets were resuspended in 200 μ l TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). For yeasts, 50 mg of 425–600 μ m size-fractionated glass beads (Sigma) were added to the cell suspension. Then 100 μ l Tris-saturated phenol (pH 8.0) was added to these tubes, followed by a vortex-mixing step of 60 s for bacteria, 120–200 s for yeasts, to lyse cells⁹. The samples were subsequently centrifuged at 13 000g for 5 min at 4 C to

separate the aqueous phase from the organic phase. 160 μ l upper aqueous phase was transferred to a clean 1.5 ml tube. 40 μ l TE buffer was added to make 200 μ l and mixed with 100 μ l chloroform and centrifuged for 5 min at 13 000g at 4 C.¹⁰ Lysate was purified by chloroform extraction until a white interface was no longer present; this procedure might have to be repeated two to three times. 160 μ l upper aqueous phase was transferred to a clean 1.5 ml tube. 40 μ l TE and 5 μ l RNase (at 10 mg/ml) were added and incubated at 37 C for 10 min to digest RNA. Then 100 μ l chloroform was added to the tube, mixed well and centrifuged for 5 min at 13 000g at 4 C. 150 μ l upper aqueous phase was transferred to a clean 1.5 ml tube. The aqueous phase contained purified DNA and was directly used for the sub-sequent experiments or stored at 20 C. The purity and yield of the DNA were assessed spectrophotometrically by calculating the A260/A280 ratios and the A260 values to determine protein impurities and DNA concentrations. PCR Condition no stage

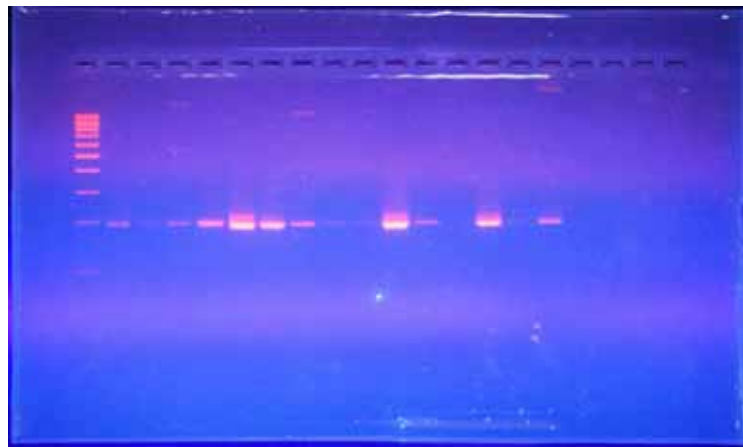
Primers:

Gene	Primer Sequence		Product size (bp)	References
<i>bla</i> NDM-1	F	'5-GATTGCGACTTATGCCAATG-3'	189	6
	R	5'-TCGATCCCAACGGTGATATT-3'		
<i>bla</i> NDM-2	F	'5-CACCTCATG TTTGAATTC GCC-3'	500	9
	R	5'-CTC TGT CAC ATC GAAATCGC-3'		

Result

NDM-1: Successful PCR amplification was confirmed by agarose gel electrophoresis. Agarose gel was prepared by dissolving 1.6 gm of agarose powder in 80ml of TBE buffer (pH:8) in concentration 0.02 in boiling microwave, allowed to cool to 50oC and ethidium bromide at the concentration of 0.5mg/ml was added. The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel, 5 μ l of DNA sample was transferred into the wells in agarose gel, and in one well we put the 5 μ l DNA ladder mixed with 1 μ l of loading buffer. This result in agreement with³

43	Staphylococcus aureus
99	E.coliFecales
82	E.coli
94	E.coli
3	Klebsiella
9	E.coli
97	Staphylococcus Haemolyticus
5	Staphylococcus aureus
86	E.coli
59	Pantoea
103	E.coli
88	Staphylococcus Hemolytic
53	Streptococcus
18	Escherichia coli
73	Pseudomonas
	Ladder

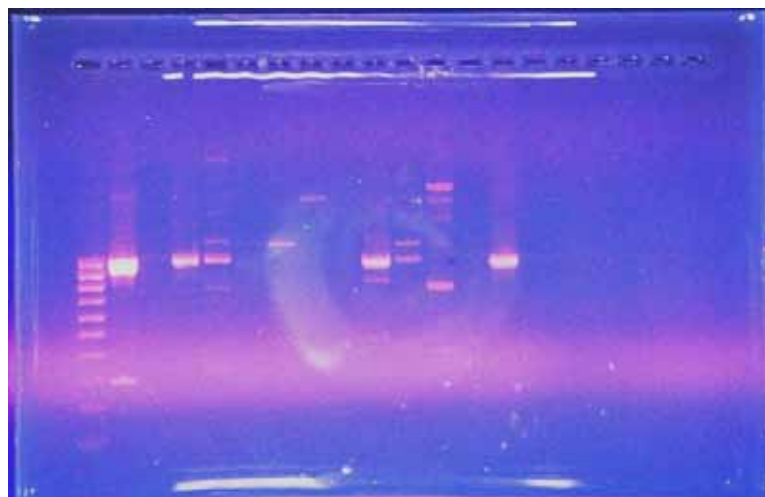


The above figure(1) shows the PCR result of the NDM1 gene, which was tested for its absence in 15 samples of which 14 showed positive samples containing the NDM-1 gene. The electric current was allowed at 100 volt for 60min. UV transilluminater was used for the observation of DNA bands, and gel was photographed using a digital camera. This result in agreement with ⁵

NDM-2: Successful PCR amplification was confirmed by agarose gel electrophoresis. Agarose gel was prepared by dissolving 0.8 gm of agarose powder in 80ml of TBE buffer (pH:8) in concentration 0.01 in boiling microwave, allowed to cool to 50oC and ethidium bromide at the concentration of 0.5mg/ml was added. The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel, 5µl of DNA sample was transferred into the wells in agarose gel, and in one well

we put the 5µl DNA ladder mixed with 1µl of loading buffer.

59	Pantoea
5	Staphylococcus aureus
9	E.coli
94	E.coli
103	E.coli
43	Staphylococcus aureus
86	E.coli
53	Streptococcus
97	staphylococcus Haemolyticus
82	E.coli
88	Staphylococcus Hemolytic
3	Klebsiella
18	E.coli
73	Pseudomonas
99	E.coliFecales
	Ladder



The above figure(2) shows the PCR result of the NDM-2 gene, which was tested for its absence in 15 samples of which 14 showed positive samples containing the NDM-2 gene. The electric current was allowed at 80 volt for 70min. UV transilluminater was used for the observation of DNA bands, and gel was photographed using a digital camera. This result in agreement with.³

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Conflict of Interest: All authors in this work have approved it for publication in your journal .

Date: All data were analyzed during this work are included in the manuscript.

Ethics Statement: The work dose note contain any study with animals performed or human participant by any of the author .

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