Study of Ceftriaxone Resistance Gene in Bacteria Isolated from Children with Nephrotic Syndrome

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Abstract

Objective: This study aim to study bacteria isolate to from Nephrotic syndrome children have septicemia by detect isolated bacteria resistance genes to antibiotics common for children (Ceftriaxone).

Method: In the Pediatric teaching hospital Center for Nephrology /Karbala Health Directorate, a five - milliliter sample of venous blood was collected from 116 Nephrotic syndrome children patient and 42 healthy children in age (months to 15 years). There are significant blood culturing procedures that was followed, and several analyses performed, the resistant genes for ceftriaxone were identified from positive blood culture sample by PCR Technique.

Results: Bacteria isolated was Acinetobacter bumanii complex, Staphylococcus warnerii, and Bacillus cerus. All of them had resistance genes for β-lactamase drug. Acinetobacter bumani which resistance to ceftriaxone showed that blaSHv and blaCTX_M were found and appeared in band 795bp and550 bp respectively in this species of Acinetobacter bumani complex. Staphylococcus warneri which resistance to ceftriaxone showed that blaZ and was found and appeared in band 173 bp. Bacillus cerus which resistance to ceftriaxone showed that genes blaA1 was found and appeared in band 680 bp.

Keywords: NS, β-lactamase genes, Bacterial resistence, PCR

1. Introduction

One kidney condition called Nephrotic Syndrome (NS) is linked to increased permeability across the glomerular purification barrier. Hypoalbuminemia (2.5 g/dl), edema, and hyperlipidemia all appear to be indications of hypoalbuminemia. Heavy proteinuria is defined as >3.5 g/24 hr in adults or 40 mg/m2 induced by HR in children [1].

The two most prevalent idiopathic kidney sicknesses are Minimal change nephrotic syndrome (MCNS) & Focal segmental glomerulosclerosis (FSG) which result in childhood nephrotic syndromes (FSGS) [2] The most frequent primary glomerular disorders in Iraq are FSGS, which are followed by mesangial glomerular nephritis and minimum change disease (MCD) [3]. Depletion of serum proteins inside the urine causes NS problems as a direct result of changes in plasma protein concentrations or as a direct cause of cellular dysfunction. Several infections, thrombosis, circulatory disease, anemia (loss of Hb), hypovolemic crisis & acute renal failure are disorder complications [4]. Infection is a major cause of mortality and death in children of renal disease. Patients with SSNS are more prone to bacterial infections, and various infections can trigger relapses, steroid resistance, and even disease onset [5].

Antibiotic resistance is a serious public health issue, and rapid detection of resistant isolates requires the use of diagnostic bacteriology labs. The performance, affordability, and usability of future technologies are evaluated against the "gold standard" of the use of phenotypic tests to identify susceptibility or resistance. There are various molecular techniques for determining resistance, and both academic institutions and reference labs frequently employ them. On the other hand, getting a footing in diagnostic laboratories is proving more challenging. However, if widely utilized in a diagnostic setting, these techniques would have a greater effect on patient care and are valuable for infection control efforts, for example, by quickly identifying individuals who were colonized by resistant bacteria.

It is obvious that rapid molecular testing detection of a specific resistance mechanism would enable clinicians to avoid potentially ineffective treatment alternatives at the outset [6]. Despite of the fact that today live in an era where advanced and new tools for elucidating disease underlying mechanisms and molecularly building new drugs are possible. We have a long way to go, infectious diseases remain one of the world's greatest serious health issues ,the rise of multi - drug resistance and unpleasant side effects are the main drawbacks of conventional antibacterial agents [7]. Over the last few decades, dangerous, antibiotic-resistant bacteria have become progressively public [8]. One of the key factors contributing to the rapid spread of AMR among bacterial populations is the presence of resistance-granting genes in plasmids or other highly genetic elements that may be independently duplicated and transmitted from one bacterial species to another. A freshly discovered antimicrobial drug is frequently effective and licensed for therapeutic use for months or years before clinically relevant resistance begins to appear [9].

A method that is frequently used in fundamental and biological sciences is the polymerase chain reaction (PCR).

doi.org/10.31838/hiv22.02.293 Received: 10.04.22, Revised: 11.05.22, Accepted: 12.08.22

To amplify specific DNA segments for use in research and/or medicine, a laboratory technique called PCR is performed [10]. Its sensitivity is based on enzyme-based amplification, and its specificity is based on sequence hybridization. A sequence of temperature cycles is typically repeated 20 to 40 times in PCR .Each cycle begins with the denaturation of DNA duplexes, followed by the hybridization of two DNA primers with a DNA polymerase. Each cycle separately doubles the number of target DNA molecules (Exponential Amplification), and then after n cycles, 2n copies might theoretically be created [11]. Bacteria cause approximately 90% of all HAIs [12]. Hospital Acquired infection (HAIs) are critical problematic in the industrial world, taking incidence rates of 5% and 7.1 % in the US and EU, respectively. The problem is much worse in developing states, where sterilized applies are less severe, with an estimated incidence at 15.5 percent [13].

Furthermore, the patients who are often immunocompromised and are the target of these infections have higher rates of death than those who have a healthy immune system. Infections caused by resistant bacteria have a much higher risk of fatality as infections caused by antibiotic-sensitive bacteria [14].

The proposal research aims to isolate the resistance bacteria from nephrotic syndrome children's patients who used ceftriaxone to treat the infection and detect resistance gene.

2. Materials and Methods

From November 2021 to April 2022, a case control study for pediatric patients which have Nephrotic syndrome was done. In Karbala teaching hospital for children, Karbala pediatric hospital/Karbala Health Directorate, one hundred and sixteen pediatric patients were diagnosed with Nephrotic Syndrome and forty-two as healthy pediatric persons. All of the patients were children, age up to fifteen (15), of both sexes, with Nephrotic Syndrome.

Blood was drawn from an arm vein and placed in blood culture bottles. The BacT/ ALERT® 3D system (bioMeriéux, Marcy l'Etoile, France) was used for the first examination of the blood cultures. The bacteria were collected, inoculated on blood agar (BAP; Asan Pharmaceutical Co., Ltd., Seoul, Korea) and MacConkey agar (Becton Dickinson, Sparks, MD, USA), and then incubated for 48 hours at 35°C in a 5 percent CO2 environment [15].Then collected positive blood culture and identified By Vitec System. Bacteria was isolated were Acinetobacter bumanii complex, Staphylococcus warnerii and Bacillus cereus

Identification of resistance genes to ceftriaxone in isolated bacteria.

Molecular detection: The thermal lysis method was used to extract the DNA for monoplex-PCR [16]. The primers used in this research were mentioned in table 1 bellow.

Table 1: primers used in this research.

Organism	Target gene	Primer Sequence 5´- 3´	Size (bp)	Temp 0C	Reference
Acinetobacter bumani complex	blaSHV-F blaSHV-R	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG	795	59.1 58.6	[17]
	blaTEM-F blaTEM-R	ATGATGATTCAACATTTCCG CCAATGCTTAATCAGTGAGG	858	52.2 55.2	[17]
	blaCTX-M-F blaCTX-M-R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550	58.5 58	[17]
Staphylococcus Warnie	blaZ-F blaZ-R	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173	59.6 57.2	[18]
Bacillus cereus	blaA1-F blaA1-R	CATTGCAAGTTGAAGCGAAA TGTCCCGTAACTTCCAGCTC	680	52.2 56.7	[19]

As previously stated, 2 whole cell lysates DNA were used separately in 25 PCR-master mix with amplification primers for each isolate in monoplex-PCR (Thermo cycler Bio base). The following were the PCR amplification conditions: The primers were made by annealing at 56°C for 1.5 minutes, extending for 95°C for 1 minute, and then final extending at 95°C for 10 minutes. Denaturation at 95°C for five minutes was followed by 35 cycles of denaturation at 95°C for one minute.

Steps were used to analyze the PCR products by agarose gel electrophoresis (Bio base) then UV Transilluminator (Bio base) was used to see PCR products.

3. Results and Discussion

From 116 nephrotic syndrome children 6 blood culture were positive and identification resulted these species bacteria isolated from Nephrotic Syndrome patient children in Table 2.

Table 2: The bacteria isolated from Nephrotic Syndrome patient children

Genus	Species	Number
Acinetobacter	Bumanii complex	2
Staphylococcus	Warnerii	2
Bacillus	Cerus	2

The widespread Gram-negative coccobacilli Acinetobacter bumanii (A) species frequently cause nosocomial infections, including urinary system and cellulitis, ventilator-associated pneumonia, and catheter-associated bacteremia [20]. In other studies they reported these bacteria were resistance to Ceftriaxone, Cefotaxime ,Ceftazidime, Ciprofloxacin, Gentamicin, Levofloxacin, Tobramycin, Piperacillin, Piperacillin-tazobactam, Ticarcillin, Ticarcillin-Clavulanate, Imipenem. Doxycycline, Amikacin, Trimethoprim, and its lone intermediate, Tetracycline, seemed to make them more sensitive [21].

Coagulase-negative A variety of illnesses can be brought on by the opportunistic microbe Staphylococcus warneri, especially in those who have indwelling medical devices [22]. The research points to S. warneri as a possible cause of clinically significant pediatric bacteremia [23].

Other studies found that this bacteria species was sensitive to oxacillin, penicillin G, clindamycin,

erythromycin, gentamicin, tetracycline, ciprofloxacin, TMP/SMX, vancomycin, and linezolid. Novobiocin & polymyxin B were both toxic to the strain [24]. While Bacillus cerus Antibiotic susceptibility from blood isolates in study of Keda et al. also showed it is sensitive to Vancomycin 100%,Imipenem 100%,Gentamicin 100%,Amikacin 100%,Linezolid 100%,Chloramphinicol 100%,Rifampin 100% ,Levofloxacin 89.7%,Clindamycin 34.5%,Erythromycin 62.1%,Cefazolin 51.7%,Daptomycin 36.4%,Cefotaxime 81.8%,Ampicilin /sulbactam4%, Ampicillin 0%, Ceftazidime 0% [25].

In recent years, ceftriaxone has been a component of therapy regimens used to treat some of the most virulent bacterial infections. However, rising bacterial resistance to third generation cephalosporin antibiotics like ceftriaxone and others has created severe clinical concerns. As a result of advancements in nanotechnology, better targeting and lower drug consumption, the concept of nanotherapeutics is becoming a tenable reality [26].

Accinitobacter bumanei complex resistance genes for ceftriaxone

In figure1, the results of detected genes of Acinetobacter bumani which resistance to ceftriaxone by PCR then appeared by gel electrophoresis showed that blaSHv and blaCTX_M were found and appeared in band 795bp and550 bp respectively in this species of Acinetobacter bumani complex isolated from child female with nephrotic syndrome who had bacteremia.

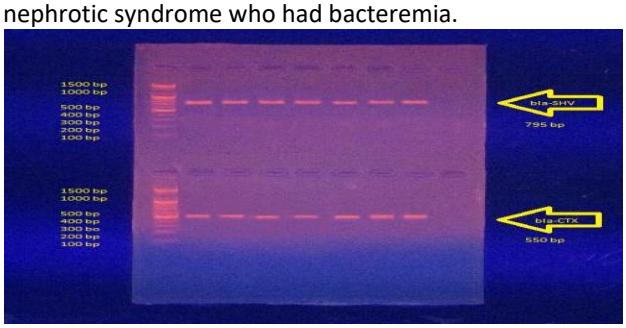

Figure 1: UV results band for gell electrophoresis in Acinetobacter bumani complex resistance genes

Acinetobacter baumannii, which is linked to nosocomial infections, is one of the top six drug-resistant bacteria. Drug resistance has evolved as a result of the widespread usage of the -lactam antibiotic class, creating a serious therapeutic quandary. Extended spectrum -lactamases (ESBLs), one of the more recent -lactamases, have become a major contributor to cephalosporin resistance [27].

The most important -lactamase variations are those for CTX-M, SHV, TEM, VEB, GES, PER, TLA, and OXA, which have widened their substrate specificity against ceftazidime, cefotaxime, and ceftriaxone. Additionally, a lot of clinical infections contain several -lactam genes. These genes are readily transmissible because of their plasmid connection [28].

Asia is particularly impacted by expanded spectrum manufacturers of -lactamases that cause the phenotype of multidrug resistance There has been evidence of the community-association of ESBL-producers in several research [29].

Resistance in A. baumannii has been linked to mutations in penicillin-binding proteins (PBPs), alterations in membrane permeability, and chromosomal or plasmid-borne cephalosporinases produced by -lactamases (bla-genes), blaTEM, blaSHV, and blaCTX-M [27].

The result in this study was similar to study of Abrar et al., which also did not find blaTEM encode gene in 8 isolated Acinetobacter bumani species in Pakistan.

All 73 A. baumannii isolates were discovered by Smiline et al. [27] to be resistant to several cephalosporin antibiotics, and blaTEM, blaSHV, and blaCTX-M molecular analysis showed PCR positivity of 57.5 percent (n = 42) for blaTEM, blaSHV, and blaCTX-M, 6.8 % (n = 5) for blaSHV, and 6.8 % (n = 5) for blaCTX-M, However, none of the strains had blaCTX-M. Three isolates (4.1 percent) included blaTEM & blaSHV. DDST positive isolates had 30.1 percent (n = 22) and 1.4 percent (n = 1) blaTEM & blaSHV, respectively, whereas CDM verified strains had 32.9 percent (n = 24) and 2.7 percent (n = 2) blaTEM and blaSHV positive isolates [27].

Staphylococcus warnei resistance gene for ceftriaxone

In figure 2 the results of detected genes of Staphylococcus warneri which resistance to ceftriaxone by PCR then appeared by gell electrophoresis showed that blaZ and was found and appeared in band 173 bp in this species of Staphylococcus warneri isolated from female child with nephrotic syndrome that had bacteremia after appendix surgery.

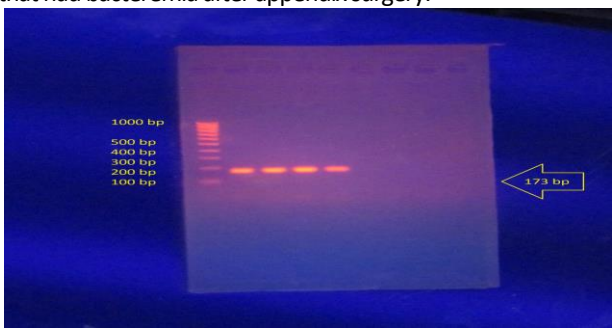

Figure 2: UV results band for gell electrophoresis for Staphylococcus warnerii resistance gene

Especially in immunocompromised people, coagulase-negative staphylococci (CNS) have become one of the most frequent bacteria to cause nosocomial infections worldwide. Infections vary from superficial wound involvement to deeper soft tissue infections. Clinically, these infections are milder than those caused by Staphylococcus aureus & Candida sp. However, the increased antimicrobial resistance over the past few decades has made therapy more challenging. There are now much more strains of bacteria that are resistant to penicillin, oxacillin/methicillin, ciprofloxacin, clindamycin, erythromycin, and gentamicin [30]. CNS may easily obtain antibiotic resistance genes via conjugative plasmids that can spread these determinants between genera and species. In this way, CNS are a problematic group in hospitals because they can be pathogenic to humans or reservoir genes for more deadly bacteria. Enzymatic antibiotic inactivation (e.g., expressed by the genes blaZ, ermA, ermB, ermC, & aac apD), active antibiotic removal from the cell The two most frequently found resistance mechanisms in Staphylococcus are decreased antibiotic binding affinity to the medication and efflux mechanisms (such as pumps) [31].

Two CNS strains (one of S. epidermidis and one of S. warneri) were discovered to be resistant to oxacillin but to lack mecA by Martineau et al. Nitrocefin testing was used to further characterize these two strains. The presence of blaZ and a positive response to the nitrocefin test for both bacteria revealed that they were -lactamase producers [32].

In the research of Pedrose et al., which analyzed some coagulase negative staphylococci for resistance genes showed r S. warneri: blaZ (71.4%), mecA (42.8%); vanA (0%).This agreement with this study results.

Bacilus cerus resistance genes for ceftriaxone

In figure 3 the results of detected genes of Bacillus cerus which resistance to ceftriaxone by PCR then appeared by gell electrophoresis showed that genes blaA1 was found and appeared in band 680 bp in this species of Bacilus cerus which isolated from male child blood with nephrotic syndrome who had bacteremia. (This bacteria isolate after give positive culture was don't identified by VITEK apparatus even after repeated test .so diagnosed it by PCR).

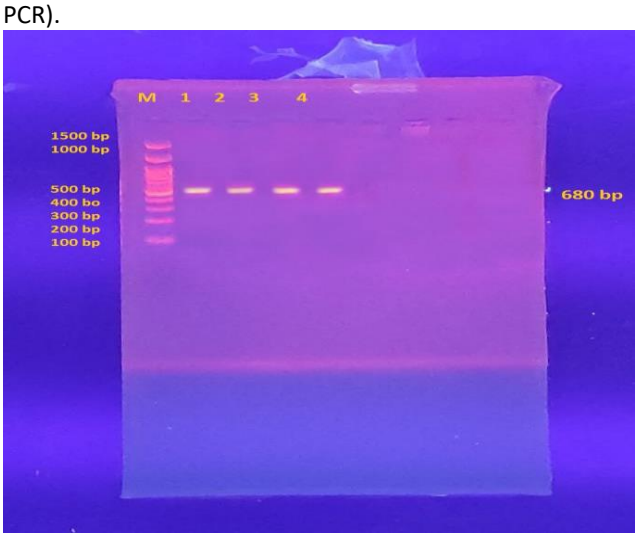

Figure 3: UV results band for gell electrophoresis in Bacillus cerus resistance gene

Members of the genus Bacillus cereus are spore-forming microorganisms that are frequently linked to intestinal illnesses & food poisoning. Additionally, some strains of the group can cause bacteremia in humans, especially in people with impaired immune systems [33].

Immunosuppressed people, patients having surgery, intravenous drug, & patients having indwelling catheters are the most commonly infected people. In most cases, B. cereus catheter-related infections are induced by the production of biofilm on biomedical equipment [34]. B. cereus group sources in the hospital environment includes air filtration & ventilation equipment, fiber-optic bronchoscopy equipment, intravenous catheters, & alcohol-based hand wash solutions. In recent seasons, it has been proposed that B. cereus strains obtained from an external cause (food, water, environment) can enter the gastrointestinal system, produce mucosal necrosis, & spread to other organs via the bloodstream [35].

Bianco et al study.'S of 17 isolated Bacillus cerus of blood patients with bacteremia found beta-lactamase resistance genes: BLA-1, BLA-2, and blaZ 12 in 100 percent (17/17) and 6 percent (1/17) of isolates, respectively. Eight isolates from beta-lactam antibiotic class were resistant to ceftriaxone, while nine isolates exhibited intermediate resistance. This agreement with this study which found blaA1 gene in isolated Bacillus cerus which also intermediate resistance to ceftriaxone after susceptibility test.

4. Conclusion

Most bacterial species that causes blood infections to nephrotic syndrome children of Karbala were nosocomial bacterial infection.

Some bacterial isolated was resistant to ceftriaxone.

B-Lactam resistance genes (especially ceftriaxone resistance genes) were found in all bacteria isolated from NS children have bacteremia.

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