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Frequency of genotypically confirmed VRSA and MRSA Among Children with Urinary Tract Infection.

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Abstract

Background:-Urinary system Infections are common bacterial-infections in children. *Staphylococcus* Spp. especially *Staphylococcus aureus*-methicillin-resistant, (MRSA) strain is a important bacteria pathogen that causes many infections. Vancomycin-resistance *Staphylococcus aureus* strain (VRSA) among "MRSA" isolates is an emerging threat associated with presumptive treatment of these infection-s. Therefore, the aims of these study to determined the frequency of *Staphylococcus aureus* (S. aureus) isolates in children with UTI and detect *mec*A (methicillin resistance) and *vanA* (Vancomycin resistance) genes among the isolates that responsible of various antimicrobial resistance. **Methods:-** A total of (850) urine samples from the children that suspected to UTI were collected. of *Staphylococcus aureus* identified using culture methods and biochemical tests then isolates confirmed by PCR (16S rRNA). MRSA screening was performed using the antibiotic cefoxitin disks and for screening of VRSA using the vancomycin broth minimal inhibitory concentration (MIC).Then confirmed by PCR using *mec*A and *van*A gene. **Results:** A total of (265/850) *Staphylococcus* Spp. (31.2%) were isolates from patients had urinary-tract infection. Out of (265) *Staphylococcus* Spp. (115) isolates was *Staphylococcus aureus* (43.4%) confirmed genotypically using (16S rRNA). A total of (96/115) *S. aureus* isolates (83.5%) determined methicillin resistance phenotypically. Out of (96) MRSA isolates, only (83) isolates carry *mecA* and determined MRSA by PCR. A total of (32/115) *S. aureus* isolates (27.8%) determined vancomycin resistance phenotypically. Out of (32) VRSA isolates, only (19) isolates carry *vanA* and determined VRSA by PCR.

Key words: S. aureus, resistance, MRSA, VRSA, mecA, vanA

1. Introduction

Urinary-system infection is widespread bacterial-infection in childr-en, rapid diagnosis and suitable intervention are necessitous to decrease the illness associated with this condition [1-2]. It has been assessed that around (7.8%) of girls and (1.7%) of children (males) at the age of seven, they had a urinary-system infection, and at the age-group of sixteen, (11.3%) of female-(girls) and (3.6%) of male-(boys), they had a urinary-system infection [1-3]. Blood-borne spread of disease, an un-common cause of bladder-infection, may be caused by *Staphylococcus aureus (S aureus) [4]*. S aureus, which is a Gram-+ve bacterium, adapted in different environments due to theire metabolic variation and pharmic resistance [5-6]. Across years ago, S aureus has improved many antimicrobial resistance strategies, this makes S.aureus difficult to cure with traditional antibiotics, including "βeta-lactamase production", "methicillin resistance (MRSA)", "vancomycin resistance (VRSA)", "macrolide", "aminoglycoside and quinolone resistances", and formation of biofilm [6-7]. The spread of ("MRSA") through developing countries has been a major difficulty in realizing effective management [8]. βeta-lactama antibiotic resistance by 'MRSA' strain is associated with *mec* gene (*mec A, mec B* or *mec C*) which is found on staphylococcal chromosomal_cassette-mec (*SCC mec*) [9-10-11-12]. These genes not just linked with resistance of Staphylococcus aureus to methiicillin but also to other antibiotic classes such as "Macrolides", "Lincosamides", "Streptogramins B", "Tetracyclines", and "Aminoglycosides" [10]. The World Health Organization (WHO) in 2017 recorded VRSA as a "high priority antibiotic-resistant pathogens" [13]. Vancomycin-resistance in *S. aureus* is predominantly awarded by *vanA* operon which is carried on transposon "Tn-1546", and additional *van* gene clusters including "*vanB, vanC, vanD, vanF, vanE, vanG vanI, vanL, vanM* and *vanN* phenotypes" [14-15]. These hereditary-components modify the structure of cell-wall, preventing vancomycin from effectively suppressing cell wall synthesis [16].

2. Martials and Methods

- **Samples collection and Bacterial Isolation**

A total of (850) midstream urine samples have been collected from children (under 12 years old) clinically suspected to have UTI. Urine samples were collected and cultured on Mannitol-Salt-Agar, Mac-Conkey agar and Blood-Agar media separately to bacterial isolation. The observed colonies after 18 hour of incubation at (37 ^{o}C) were selected and further sub-culture to mannitol-salt-agar, followed by bio-chemical tested that are routine for diagnosis of *S.aureus* which includes; "catalase test, coagulase test, DNase on Dnase-agar medium, gelatin hydrolysis and oxidase test", then isolates confirmed by PCR (16S rRNA).

- **Screening of Methicillin resistant S. aureus**

Antibiotic disks (cefoxitin) are used to screen for MRSA according to CLSI guidelines. The in-hibition zone less than ('22 mm') around the growth of *S. aureus* is considered MRSA. Then confirmed by PCR using *mecA*.

- **Screening of Vancomycin-resistant S. aureus**

A vancomycin broth (VB.) 'Minimum-Inhibitory-Concentration' (MIC) >= (two µg/ml) is considered susceptible, (two-four µg/ml) MIC is considered intermediate, and MIC more or equal to (sixteen µg/ml) is considered resistant to vancomycin. *S aureus* "ATCC 29213" MIC of VB. with a value-of (0.5--2.0 µg/ml) was used as a control strain to determinate the efficacy of vancomycin. Then confirmed by PCR using *VanA*.

- **Genes amplification of *vanA* and *mecA* using PCR**

Two sets of primers are used to amplified *vanA* and *mecA;* used
F: [5′-ACTGCTATCCACCCTCAAAC-3′]

R: [5′-CTGGTGAAGTTGTAATC TGG-3′]"(Amplicon size-163 bp) for *the mecA* gene [17]. And;
F: ["5′-ATGAATAGAATAAAAGTTGC-3′]

R: ["5′-TCACCCCTTTAACGCTAATA-3′] "(Amplicon size-1100 bp) for *the vanA* gene [18].

Statistics analysis

The data were entered and examined using the Statistical program package for the Social Science (SPSS) version-24. A p-value "≤ 0.05" is considered statistically significant.

3. Results

A total of (265/850) *Staphylococcus* Spp. (31.2%) were isolates from patients had urinary-tract infection. Out of (265) *Staphylococcus* Spp. (115) isolates was *Staphylococcus aureus* (43.4%) confirmed genotypically using (16S rRNA).

- **Determination of Methicillin (MRSA) and vancomycin (VRSA) resistance *S. aureus* Phenotypically and genotypically**

A total of (96/115) *S. aureus* isolates (83.5%) determined methicillin resistance phenotypically. Out of (96) MRSA isolates, only (83) isolates carry *mecA* and determined MRSA by PCR (Table 1). A total of (32/115) *S. aureus* isolates (27.8%) determined vancomycin resistance phenotypically. Out of (32) VRSA isolates, only (19) isolates carry *vanA* and determined VRSA by PCR (Table 1). And 12.5% of isolates have *mec*A and *van*A gene.

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Table_1: Frequency of VRSA & MRSA among S aureus isolates.

Isolates	N. Frequency	Total	P- Value ≤ 0.05
***Staphylococcus* Spp.**	256 (31.2%)	850 (100%)	
***S. aureus* (16srRNA)**	115(43.4%)	265 (100%)	
MRSA (phenotypically)	96 (83.5%)	115 (100%)	
MRSA (*mec A*)	83 (72.2%)	115 (100%)	
VRSA (phenotypically)	32 (27.8%)	115 (100%)	
VRSA (*Van A*)	19 (16.5%)	115 (100%)	
MRSA have ***VanA***	12 (14.5%)	83 (100%)	

4. Discussion

The acquisition of penicillin-binding proteins "PBP2a", which are encoded by *mecA* gene, is associated with the expression of methiciillin-resistance (MR) in S aureus [19]. Despite the fact that S. aureus is a common microbiota of the human body's, it has an ability to cause a various of illness, from minor skin infections to more severe diseases effecting on the body as a whole [20]. In this study, we observed MRSA around (83.5%) of *S. aureus*. Among "MRSA", the *mec*A gene was observed in more-than (70%) of the isolates and among VRSA, the *Van*A gene was observed in (16.5%) of the isolates. Al-though the diagnosis of *S aureus.* in this study was comparatively similar in the study conducted previously in Dhi Qar Governorate/Iraq [21]. While other study in Dhi Qar detected (32.1%) of the isolates was MRSA, whereas VRSA was found in (35.4%) of isolates [22]. Methicillin resistance staphylococcus is a word health risk, as indicated by the high rates of anti-microbial non-susceptibility reported in countries, such as Turkey-(21%), Gaza (82.3%) and Iran (71.9%) [23][24]. Rates of occurrence of VRSA strains differ globally: (sixteen %) in Africa, (five %) in Asia, and (one %) in Europe, (four %) in America-North, and (three %) in America-South [25]. The possible reasons for the higher rate of VRSA could possibly be unsanitary conditions, lack of proper supervision of noso-comial infections and unappropriated use of antibiotics in health-care centers. In many developing countries the anti-microbial drugs are easily available over the counter [26][27]. However, the limited resources available for testing may cause a wrong representation of VRSA rates in developing countries, and the total numbers of tests performed may not exactly reflect the actual number of infections [28].

5. Conclusions and recommendations

The current work revealed a high rates of MRSA has been reported among children with UTI and the VRSA isolates were reported in our recent analysis. In this study, the rates of MRSAspread were higher than those of VRSA. Out of 83 MRSA isolates (72.2%) was carry mecA gene and out of 32 VRSA isolates (16.5%) was carry vanA lead to high resistance to antibiotic. An urgent need to control the rapid emergence of vancomycin resistance is evident, and this can be achieved through various measures such as promoting the responsible use of antibiotics and hygiene care in children is essential for preventing infections and promoting overall health. To the best of our knowledge, this is the first study in Wasit province to report VRSA among children with UTI.

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