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Frequency of MecA, Van A and Van B Genes in *Staphylococcus aureus* isolates among pediatric clinical specimens in Khartoum Hospitals 2017

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Abstract:

Methicillin resistant Staphylococcus aureus (S. aureus) (MRSA) infections strains is increases number in global health threat. Vancomycin is one of the very limited options in treating such infections. The emergence of vancomycin-resistant S. aureus (VRSA) is therefore a great concern in clinical settings. During recent years, the incidence of vancomycin-intermediate S. aureus (VISA) and vancomycin resistant S. aureus has increased in the world. This study was conducted to estimate the frequency of MecA and van A, B genes in Staphylococcus aureus among children. Different clinical samples were collected from 81 children with an age range from (1-15) years old

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that was diagnosed as Staphylococcus aureus infections in Khartoum hospitals during period from October 2017 to December 2017. Out of 81 Staphylococcus aureus isolated which had been confirmed phenotypically by Biochemical method from different children clinical samples and genotypiclly by 16s gene and detect of MecA and van A and B after doing antibiotic susceptibility of Methicillin and Vancomycin resistant. Among Staphylococcus aureus identified, the antibiotic susceptibility result is 94% Methicillin resistant and 44% Vancomycin resistant .The (PCR) result is 28/50 revealed that 56% were positive for Mec A and none for van A and B. The high frequency of circulating MecA gene highlights and none of Van A, B.

Key words: MRSA VRSA, MecA and Van A, B.

INTRODUCTION:

Staphylococcus aureus infections consider major health problem in our world today. It updates itself to resist many type of antimicrobial agent .This study carry out some reasons for their resistant. *Staphylococcus aureus* is one of the most important human pathogen .it can cause wide range of illnesses from skin infection to sever condition such as sepsis, endocarditis, osteomyelitis, pneumonia...etc.(Harris *et al*., 2002).

Staphylococcus aureus have many mutant genes that cause resistant the main gene is *Mec A* gene. Theoretically the Oregon of *Mec A* gene from coagulase negative *Staphylococcus* and *Escherichia coli*. Methicillin resistant *Staphylococcus aureus* (*MRSA*) mediated by penicillin binding protein2a (PBP2a) encoded by *MecA* on mobile *Staphylococcus* cassette chromosome Mec (SCCmec) element (Reynolds, 1985).

The (*SCC Mec*) types include I, II and III. The main important is type I, II that cause multidrug resistant and Health care associated (Ito, 2009). The second emerging gene is *Van* genes there are many type of *Van* gene but here we talk

about the more comment types in the world however, reports of vancomycin Resistant for *Staphylococcus aureus* isolates with reduced susceptibility first alarm (Perichon *et al*., 2009) in Japan in 1996, (Hiramatsu, 2008), ***Van A and B*** originated from *Enterococcus* spp. The Vancomycin resistant glycopeptides were mediated by *Van* gene altering drug target from D-alanine to D-lactate (Courvalin, 2006).

MATERIAL AND METHODS:

The current study was performed from the period of October 2017 to December 2017. Informed consent was obtained from children the age range from (1-15) years old. The tested samples were include (81) from different clinical samples (Swab-Urine - Blood) which had been sub cultured in mannitol salt agar in aerobic condition at 37c. Then further identified phenotypically by gram stain and biochemical method (Catalase test, coagulase test, DNA-se) and to conforming the identified samples is *S, aureus* we conduct molecular identification by 16s gene and from the (81) there were (50) Positive to be *Staphylococcus aureus*. Then we Carry antibiotic susceptibility test (Kirby Bauer) disc diffusion method (1 *µg* Oxacillin, 30 *µg* Vancomycin) that were ably according with guideline of clinical and laboratory standard institute (Wayne, 2012).

The strains subjected to further genotypic investigation for *MecA* and *van A, B*, The DNA was extracted by modified boiling method. PCR was did to amplification of four genes 16s rRNA Forward 5AGTTTGATCCTGGCTCAG3 Reverse 5AGGCCCGGGAACGTATTCAC3 1500 bp (Woo *et al*., 2003). *MecA* Forward: 5TGGCTATCGTGTCACAATCG3 reverse: 5CTGGAACTTGTTGAGCAGAG3 310 bp (Dias *et al*., 2004).

Van A Forward: 5ATGAATAGAATAAAAGTTGC3 reverse: 5TCACCCCTTTAACGCTAATA3 1032 bp *and Van B*

Forward: 5GATATTCAAAGCTCCGCAGC3 Reverse: 5GGTATCTTCCGCATCCATCA3 368 bp (Donabedian *et al.,* 2000).

PCR amplification of *Van A* gen PCR amplification conditions were initial denaturation at 94°Cfor 5 min followed by 35 cycles of denaturation at 94°C for 40s annealing 48°C for 40s, extension at 72°C for 40s and final extension at 72°C for 5 min. PCR multiplex amplification of *MecA and Van B*: PCR amplification conditions were initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40s annealing 50°C for 40s, extension at 72°C for 40s and final extension at 72°C for 5 minutes (this condition also for 16s gen). PCR products were subjected to 2% agar gel electrophoresis. The gels were stained with the ethidium bromide and examined under ultraviolet light. (Donabedian *et al.,* 2000).

RESULTS

There were 50 *S. aureus* identified in this study result of antimicrobial sensitivity *Vancomycin* resistant 44% (male 18% and female 26%), sensitive 38% (male 12% and female 26%, intermediate18% (male 8% and female 10%). *Methicillin* 94% resistant (male 34% and female 60%) and 4% sensitive (male 2% and female 2%), 2%intermediate male only Table (1).

PCR result of some 16s gene at 1500bp figure (1), PCR result of some *MRSA* isolates show *MecA* gen at 310bp figure (2) Among all *S. aureus* isolates positive for the *16s gen, 28* out of 50 (56%) were positive for the *MecA* gene(male22%female34%). None of the *S .aureus* isolates was positive for the Van A *and Van B* genes table (1).

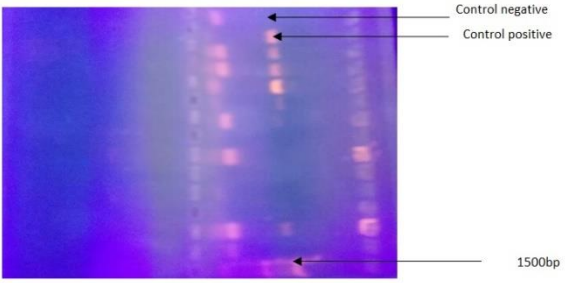

Figure (1): PCR amplification of the 16s of *S. aureus*

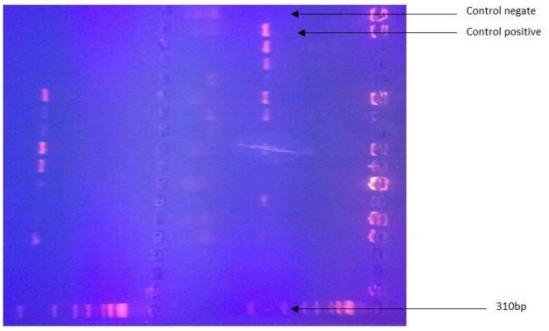

Figure (2): show *Mec*A isolate in PCR amplification

Table (1)

	Mec A		**Van A and B**	**Oxacillin AST**			**Vancomycin AST**		
	+	-		R	I	S	R	I	S
Male	11	8	Zero	17	1	1	9	4	6
Female	17	14	Zero	30	0	1	13	5	13
Total	28	22	Zero	47	1	2	22	9	19
Percentage	56%	44%	Zero	94%	2%	4%	44%	18%	38%

Key**: R**: Resistant, **I**: Intermediate, **S**: Sensitive

DISCUSSION:

The MRSA infections are serious Issue and its treatment Becoming increasingly more complicated due to emergence of various types of multidrug resistant (Sharif et al., 2013).

However, the wide usage of these drugs caused numerous methicillin resistant S. *aureus* Reports (Tong *et al*., 2012).

The alternative was vancomycin, it work as the main antimicrobial agent available to treat serious infections with (*MRSA*) (Sievert *et al*., 2002). However, reports of vancomycin Resistant for *S. aureus* isolates with reduced susceptibility first alarm (Perichon *et al*., 2009) in Japan in 1996, (Hiramatsu, 2008).

The result 94% *MRSA* and 44% *VRSA* more than the result in Iran 41, 85% (*MRSA*) 2% (*VRSA*) (Aligholi *et al*., 2012), Brazil 42% (*MRSA*) and 2.8% (*VRSA*) (Brevesa *et al*., 2015). To support this results Show another study in Sudan 76.5% (*MRSA*) and non-for van A, B (Elimam *et al*., 2001), 78% *MRSA* (Ahmed *et al.,* 2014*)* and 46.7% of *MecA* (Abdalla *et al*., 2014). However, this study cannot roll out the present of other types of *Van* genes so it will be more Advisable to further investigation to avoid the problem of emerging of *Vancomycin Resistant* of *S. aureus* in Sudan.

CONCLUSION:

High percent frequency of *MRSA* and *VRSA* isolated from children. *Van A, Van B* is not detected in *Vancomycin* resistance Staphylococcus in Sudan

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