Vancomycin Resistant *Staphylococcus Aureus* (Vrsa)

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Abstract:

Background: Vancomycin resistant Staphylococcus aureus (VRSA) are serious strains of Staphylococci that are difficult to be treated and have considerable emerging percentage in the world.

Objectives: To estimate the percentage of VRSA in Khartoum state.

Method:The different specimens were randomly collected from different patients suffering from various clinical manifestations after accepting the informed consents and cultured in number of different culture media according to each sample, then sensitivity testing is done using oxacillin(1 mcg), vancomycin(30 mcg) ,amoxyclav(30 mcg) ,erythromycin(15 mcg), clindamycin(2 mcg) and amikacin (30 mcg)discs and interpreted according to Kirby Bauer reference procedure.

Results: According to the outcome of our study, S.aureus that were resistant to vancomycin disc (30 mcg) represents 12% from total 100 S.aureus positive specimens and 3.7% from MRSA group while 15.1% from MSSA group. On the other hand clindamycin resistance represents about 10% ,Inducible clindamycin resistance forming D shape appearance 76.1% while 23.8% showed constitutive resistance.

There is a great resistance of S.aureus bacteria to amoxyclav disc(30 mcg (20/10 mcg)) with percentage of 76% while 24% nly were sensitive to the disc.

Conclusion: There is a significant percentage of S.aureus bacteria that showed resistance to vancomycin.

Keywords: Vancomycin,Staphylococcus aureus, antibiotic resistantS. aureus.

I. INTRODUCTION

Staphylococcus aureus (S.aureus)is a hospital and community-acquired pathogen that causes a broad spectrum of diseases. This pathogenicity is associated with different enzymes and toxins.[(1)]

Most *staphylococcal* infections can be easily treated with antibiotics; however, in recent years *Staphylococcus* found its way to resist the commonly used effective antibiotics; these antibiotics include macrolides, lincosamides, streptogramin, tetracycline, gentamicin, and beta-lactams particularly methicillin [(2, 3)].

Antimicrobial resistance (AMR) among *S.aureus*is a globalgrowing issue and is considered as a natural phenomenon for thesurvival of micro-organisms. Therefore, it is imperative toslow the rate of development of AMR to a level thatmaintains the usefulness of the antimicrobials[(4)] by accurate determination of bacterialsusceptibility to antibiotics This canbe achieved using a number of techniques, including thedisk diffusion method, the broth dilution assay, and the Etests [(5)]. As antibiotic resistance reducestreatment efficacy, it is a time to consider routinesusceptibility testing to guide individual patient treatmentand surveillance of antibiotic resistance [(6)].

Amoxicillin/clavulanic acid or coamoxiclav is a broad spectrum antibiotic that restore efficacy against amoxicillin-resistant bacteria that produce beta-lactamase.Reduced susceptibility to amoxycillin/clavulanic acid has become a major problem[(7)].

Then methicillin-resistant *S.aureus*(MRSA) was developed due to genetic mutation and modification in the mecA gene that leads to a lower affinity for binding beta lactams (penicillins, cephalosporins and carbapenems). This allows for resistance to all beta lactam antibiotics[(8)]. There are many options available for the treatment of methicillin sensitive (MSSA) and methicillin resistant (MRSA) *staphylococcal* infections, like clindamycin being one of the good alternatives, particularly for penicillin allergic patients [(9)]. However clindamycin resistant *S.aureus*strains began to emerge[(10)]. Although some *S. aureus* isolates are susceptible to clindamycin *in vitro*, they may not be effective *in vivo* particularly when the strain is resistant to erythromycin. This may be due to presence of inducible macrolide-lincosamide-streptogramin B resistance (iMLSB). The presence of inducible clindamycin resistance can be detected in erythromycin resistant strains by the double disk diffusion assay (D test) [(11)].

MRSA has become a major problem in most medical institutions because it is creating life-threatening situations[(12)]. Furthermore, the emergence of mutated strains of MRSA is the vancomycin resistant *S. aureus* (VRSA). VRSA is currently one of the greatest threats mankind faces because the antibiotic, vancomycin, is the last resort for treating *staphylococcal* infections[(13)]. Therefor this study was carried out to estimate the percentage of VRSA in Khartoum state.

II. METHODOLOGY

A prospective study conducted during the period from February 2017 to April 2017. The study was conducted on randomly collected samples from out and in patients from different hospitals in Khartoum state (Central and national reference laboratory, Alzarah, Aliaa and Bahry hospitals)\Sudan.

II.I. Collection and processing of samples:

A total of 284 samples werecollected including wound and ear swabs, blood , urine, abscess, and pus samples. Wound swabs, abscess, and pussamples werecultured aerobically in blood agar and macconkey agar media plus anaerobic blood agar plate, whereas ear swabs were inoculated into blood agar, chocolate blood agar and macconkey agar plates and incubated aerobically. The blood specimens were inoculated in blood culture bottle then sub cultured into two blood agar plates , one of them was incubated anaerobically while the other was incubated aerobically in addition to macconkey agar plate. Urine samples were cultured aerobically into blood agar, macconkey and CLED media .All the plates were incubated at 37°C for 24 hrs.

II.II. Identification of isolates:

The isolates were identified using colonial morphology and Gram staining to exclude the Gram negative bacteria. All Gram positive cocci were tested for Catalaseproductionto exclude all species ofstreptococci, and Coagulasetest (slide and tube method) to exclude all species of coagulase negative staphylococci. Then a total of 100pure isolates of staphylococcus aureus were confirmed using DNase test and fermentation of mannitol on mannitol salt agar media.

II.III. Antimicrobial susceptibility testing:

Sterile suspension of previously identified *S. aureus*for sensitivity testing is adjusted against (0.5)McFarland turbidity standard.Sensitivity testing was done according to Kirby-Bauerdisc diffusion technique[14]. Sterile swabswere used to inoculate the test organisms onto the sensitivityagar (Mueller Hinton agar media) [15].

Plates were dried for five minutes. Using sterile forceps, placedisks of amoxycillin/clavulanic acid (20+10 mcg) , oxacillin (1 mcg), erythromycin (15 mcg), clindamycin (2 mcg), vancomycin (30 mcg), and amikacin (30 mcg) on the plates. All plates were incubated within15 minutes after applying the disks. The diameter of inhibition zone around each disk was measured to the standard values provided by CLSI.

II.IV. Data analysis:

The level of significance between gender , age , resident , site of infection was determined against the sensitivity and resistance to each antibiotic disk using t-test. *P* value less than 0.05 was considered significant. Statistical analysis was performed by SPSS version 19.

III. RESULTS

According to the outcome of our study, there is a great resistance of *S. aureus* bacteria to the amoxyclav disc(30 mcg (20/10 mcg)) with percentage of 76% while 24% only were sensitive to the disc. 27% resistance to oxacillin disc (1 mcg) that forming MRSA group and 73% were sensitive to form MSSA group. *S.aureus* that were resistant to vancomycin disc (30 mcg) represents 12% from total specimens and 3.7% from MRSA while 15.1% were resistant from MSSA group. 88% were sensitive to the same disc. Sensitivity pattern to amikacin (30 mcg) disc showing that 13% were resistant and 84% were sensitive.

On the other hand erythromycin (15 mcg) disc showed that only 21% were resistant where 69% were sensitive. Clindamycin disc (2 mcg) having resistance percentage of 10% and 90% sensitivity percentage. Inducible clindamycin resistance forming D shape appearance 76.1% and 23.8% were constitutive resistance to clindamycin.

Table (1): Resistance and sensitivity percentages of *S. aureus* to antibiotic discs

Antibiotic disc	Resistance		Sensitivity	
	Number	Percentage	Number	Percentage
Amoxyclav disc (30 mcg)	76	76%	24	24%
Oxacillin disc (1 mcg)	27	27%	73	73%
Vancomycin disc (30 mcg)	12	12%	88	88%
Amikacin (30 mcg)	13	13%	84	84%
Erythromycin (15 mcg)	21	21%	69	69%
Clindamycin (2 mcg)	10	10%	90	90%

Table (2): Vancomycin resistance among MRSA and MSSA

Antibiotic disk	MRSA		MSSA	
	Number	percentage	number	percentage
Vancomycin(30mcg) **resistance**	1	3.7%	11	15.1%

Table (3): Inducible and constitutive Clindamycin resistance

Antibiotic disc	Inducible		Constitutive	
	number	percentage	Number	Percentage
Clindamycin(2 mcg) **resistance**	16	76.2%	5	23.8%

Figure (1): percentage of Vancomycin Resistance among Different Samples

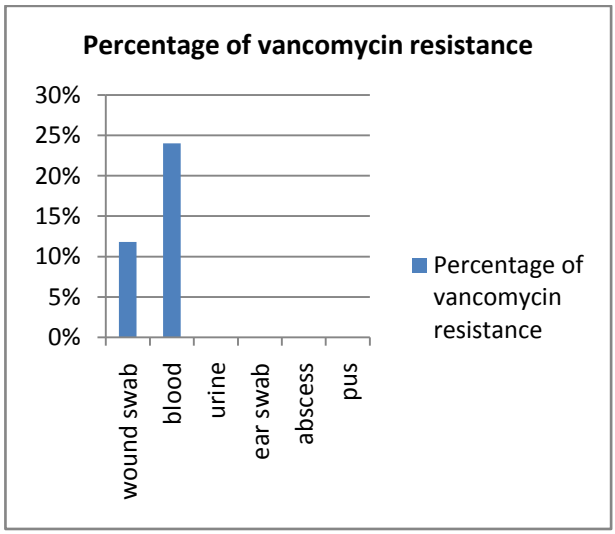


Figure (2): Percentage of Clindamycin Resistance among Different Samples

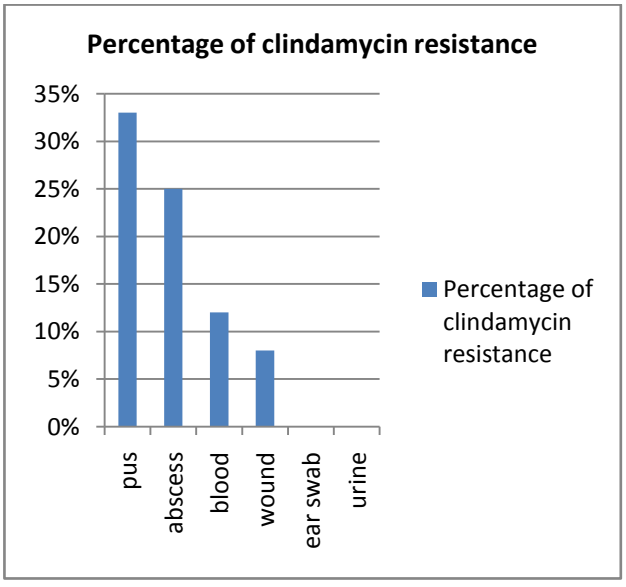


Figure (3): Percentage of Amoxyclave Resistance among Different Samples

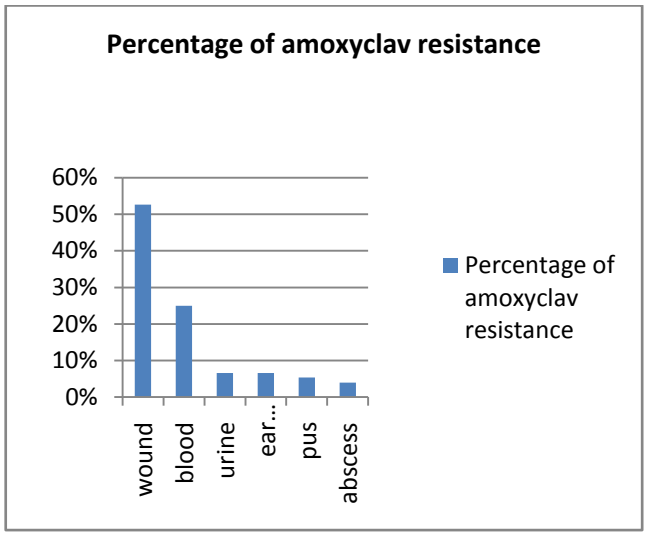


Figure (4): Total Resistance to antibiotics

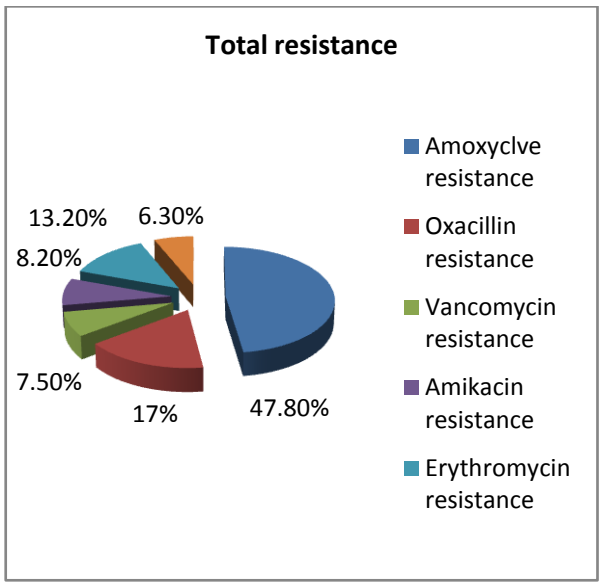
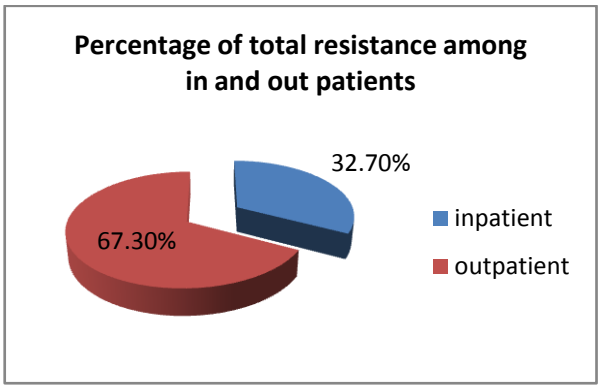


Figure (5): Percentage of the total antibiotic resistance among in and out patients.



IV. DISCUSSION

Antibiotic resistance is one of the world's most pressing public health problems. The antibiotic resistant organisms can quickly spread and so threaten communities with new

strains of infectious disease that are more difficult to cure and more expensive to treat[16]. Amoxycillin /clavulenic acid is still the first choice for the treatment of *S. aureus* infections; in this study 76% of the isolates are resistant to amoxclav. Biswajit Batabyal in India also Found that 73.3% from the isolates were amoxyclav resistant *S. aureus.*[8]

27% of the isolates are MRSA. In Iran; Fateh Rahimi and Majid Bouzari *et al* found that 29.7% of their isolates were MRSA[1]. This rate of resistance was lower than the other reports from Iran (42-90%)[17-18]. And recently MRSA infection has become highly endemic in many geo-graphical areas. It has been suggested that due to the changing pattern of antibiotic resistance in *S. aureus*, it would be wiser to have a periodical surveillance of these changes every 3 to 4 years [19, 1].

Fateh Rahimi and Majid Bouzari *et al* found Zero number of VRSA in a study carried in Iran[1]. In contrast; we have 12% of vancomycin resistant *S. aureus*.

3.7% of which are MRSA and 15.1% are MSSA. Current results were consistent with studies in Iran which have reported high prevalence (7%) of VRSA isolates [18].

Kavitha Prabhu and Sunil Rao*et al* found that (10%) isolates showed inducible clindamycin resistance, (9%) showed constitutive resistance while (8%) showed MS phenotype. Inducible resistance and constitutive resistance were found to be higher in MRSA as compared to MSSA (20%, 16% and 6%, 6%, respectively)[20]. In contrast we detected 76.2% show inducible phenomenon, 23.8% show constitutive phenotype and no isolate shows MS phenotype. Inducible resistance is higher in MSSA 81.3% as compared to MRSA representing 18.8%, further more constitutive resistance is higher in MRSA 100%.

In this study we don't found any significant relationship between oxacillin, vancomycin, amoxyclav, erythromycinand clindamycin with gender, resident, age and the site of infection (P value >0.05).

V. CONCLUSION:

Antimicrobial resistance is a globally increasing problem. And *S.aureus* infections are seriously growing problems. Resistance of this bacteria to the most effective antibiotics is the problem we really face. The effective use of antibiotics must be taken into account and the sensitivity testing must be done for the effective choice of treatment. Furthermore there was no readily identifiable risk factors for *S. aureus* infections.

VI. ACKNOWLEDGEMENT:

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VII. REFERENCES:

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