International Journal of TROPICAL DISEASE & Health
10(4): 1-5, 2015, Article no.IJTDH.20642
ISSN: 2278–1005

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Emergence of Vancomycin Intermediate *Staphylococcus aureus* (VISA) in Clinical Isolates of Methicillin Resistant *S. aureus* from South Western Region of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author DOO did the study design and wrote the protocol. Authors DOO and OATA did the statistical analysis and literature searches while analyses of study was by authors LAB and AOI. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2015/20642
Editor(s):
(1) Janvier Gasana, Department of Environmental & Occupational Health, Robert Stempel College of Public Health & Social Work, Florida International University, USA.
Reviewers:
(1) Anonymous, Universidad Autónoma Metropolitana, Mexico.
(2) Arturo Abdelnour, National Children's Hospital, Costa Rica.
(3) Ketan Vagholkar, D. Y. Patil University School of Medicine, India.
(4) S. Thenmozhi, Periyar University, India.
Complete Peer review History: http://sciencedomain.org/review-history/11442

Short Communication

Received 1st August 2015
Accepted 24th August 2015
Published 17th September 2015

ABSTRACT

Aim: Vancomycin has been widely used in the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). The emergence of vancomycin- intermediate and -resistant *Staphylococcus aureus* (VISA and VRSA, respectively) in various parts of the world has been reported. The level of vancomycin resistance phenotypically and genotypically in clinical isolates of *S. aureus* with or without methicillin resistance from south western region of Nigeria was determined.
Methods: A total of 116 non-duplicate *S. aureus* previously obtained from various clinical specimens were subjected to susceptibility testing using disc and microbroth dilution including polymerase chain reaction amplification of *mecA* and *van* genes.

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Results: The disc susceptibility testing results depict multiple drug resistance with 100% resistance to co-amoxyclav, erythromycin and gentamicin had intermediate of 39.1 and 65.2% respectively, no strain sensitive. Vancomycin showed 100% susceptibility. The minimum inhibitory concentrations, MICs of 116 *S. aureus* strains against *vancomycin showed the isolates to have* MIC_{50} of 1 µg/ml and MIC_{90} of 2 µg/ml. Five (4.3%) of the 116 clinical isolates had intermediate MIC of 4 µg/ml. These *five strains were from methicillin resistant strains and were isolated from different clinical sites and hospitals.* However, none of these strains demonstrated the presence of *van* genes, *vanA; vanB; vanC and vanH* by PCR.
Conclusion: There is high level of multiple antibiotic resistance in *S. aureus* with some MRSA also showing reduced susceptibility to vancomycin resulting in VISA. However, the VISA strains have shown no *van* gene as their mechanism of acquiring reduced susceptibility.

Keywords: Staphylococcus; vancomycin; susceptibility; van genes; Nigeria.

1. INTRODUCTION

Bacteria resistance to antibiotics have become a global menace attracting public health interest from all over the world including developing countries where the pandemic is more pronounced. This problem cut across all species of bacteria; Gram- positive and negative bacteria. In Nigeria, a number of cases have been reported for Gram negative bacteria [1] and MRSA, a prevalence of 41.4% [2]. Vancomycin became antibiotic of choice for treating serious *S. aureus* infections due to global emergence of *S. aureus* with capability of resisting various antibiotics including methicillin [3]. Simultaneous emergence of VRSA has been reported in several countries in the last two decades due to the use of vancomycin for treatment of nosocomial infection, VISA in Japan [4]; four VRSA strains from America [5]. Most of these isolates appear to have developed from preexisting MRSA infections. There has been growing resistance to vancomycin by species such as *Enterococcus*. The resistance was found to be horizontally transmitted to the *S. aureus* where they co-exist with *E. faecalis* [6]. It is noteworthy that unprecedented high level of VRSA has been reported form the northern part of Nigeria by Olayinka et al. [7] from sick people and most recent from healthy animals [8]. In both studies, the authors failed to use dilution method to determine MIC - the gold standard for determining vancomycin susceptibility [9] and genotypic detection of vancomycin resistance was also not included in their respective studies. It was in view of these short comings that we determined the level of vancomycin resistance phenotypically and genotypically in clinical isolates of *S. aureus* with or without *mecA* genes from the South Western Nigeria.

2. METHODOLOGY

2.1 Bacterial Strains

A total of 116 non-duplicate *S. aureus* that had previously been obtained from various clinical specimens submitted to the diagnostic laboratories of Medical Microbiology and Parasitology department of three major tertiary hospitals in the region for a period between January and December, 2010.

2.2 Susceptibility Testing

Inoculum of pure colonies of the same morphological types was prepared to turbidity equivalent to that of a 0.5 McFarland standard, different antibiotic discs (Oxoid, UK) were used for by disc diffusion susceptibility testing technique. MICs of vancomycin (Sigma-Aldrch, Germany) were determined by microbroth dilution method as described by CLSI [9]. *S. aureus* ATCC 25923 was used as a reference strain for both techniques.

2.3 PCR Amplification of mecA Gene

DNA lysates were prepared from colonies of the same morphology type by rapid alkaline lysis and PCR for the *mecA* gene was carried out on all the strains as previously described [10]. Briefly, the DNA template from each isolate was amplified with GeneAmp PCR system 9700 thermal Cycler (Applied Biosystems) using *mecA* primers (Table 1) in a 25 µl reaction using the cycling parameter - denaturation temperature at 94ºC for 30 sec, annealing temperature at 55ºC for 30 sec, followed by extension at 72ºC for 1 min for 40 cycles. Positive control (MRSA DNA) and negative control DNA from NCTC 6571 (Oxford *S. aureus*) were included in each batch of PCR run.

2.4 PCR Amplification of Van Genes

PCR was performed on a DNA thermal cycler described above to detect genes encoding vancomycin resistance (*vanA, vanB, vanC, vanH*) (Table 1). The cycles comprised 94℃ for 2 min for the first cycle; 94℃ for 1 min, 54℃ for 1 min, and 72℃ for 1 min for 35 cycles; and 72℃ for 10 min for the last cycle. All PCR products were resolved by electrophoresis on a 1% agarose–Tris–borate–EDTA gel containing Midori green.

3. RESULTS

3.1 Distribution of *S. aureus* and Disc Susceptibility

Table 2 shows the distribution of 116 *S. aureus* according to specimen/body site. The highest number of *S. aureus* strains was obtained from wound infection followed by urine. Ten were isolated from hospital A, 65 from hospital B, while hospital C had 41. The susceptibility pattern of all the strains against panel of antimicrobial agents including vancomycin was reported in Table 3. Briefly, the disc susceptibility results depict multiple drug resistance with 100% resistance to co-amoxyclav, erythromycin and gentamicin had intermediate of 39.1 and 65.2% respectively, no strain sensitive. Vancomycin showed 100% susceptibility though this is not a reliable method while linezolid also showed a very high potency against the strains.

3.2 Minimum Inhibitory Concentrations

The MICs of 116 *S. aureus* strains against *vancomycin showed* MIC_{50} of 1 µg/ml and MIC_{90} of 2 µg/ml; five strains (4.3%) had intermediate of MIC 4 µg/ml. These f*ive strains were from methicillin resistant strains and were isolated from different clinical sites and hospitals.* Three of these patients were suffering from chronic osteomyelitis, 2 were from hospital B while 1 was obtained from hospital C. Similarly, two were burn patients from hospitals B and C. However, none of these strains demonstrated the presence of *van* genes, *vanA; vanB; vanC and vanH* by PCR.

4. DISCUSSION

This study has shown the emergence of VISA accounting for 4.3% in the south west region and none of the strains displayed presence of *van* genes (*van*A, *van*B, *van*C and *vanC*) by PCR. Five of the strains that were MRSA displayed a

Table 1. Primers used to amplify *van* genes

Gene	Sequence (5' to 3')		Size (bp)
	Forward	Reverse	
MecA	AGTTCTGCAGTACCGGATTG	AAAATCGATGGTAAGGTTCGC	533
VanA	GGGAAAACGACAATTGC	GTACAATGCGGCCGTTA	732
VanB	ATGGGAAGCCGATAGTC	GATTTCGTTCCTCGACC	635
VanC-1	GGTATCAAGGAAACCTC	CTTCCGCCATCATAGCT	822
VanC-2, VanC-3	CTCCTACGATTCTCTTG	CGAGCAAGACCTTTAAG	439
VanH	ATGAATAACATCGGCATTAC	CTATTCATGCTCCTGTCTCC	969

Table 2. Distribution of 116 *S. aureus* strains from various body sites

Body site	No. of strain (%)	No. of strain from hospital (%)			*MecA*
		A	B	C	
Urine	25 (21.6)	2 (8.0)	20 (80.0)	3 (12.0)	16
Nasal swab	5 (4.3)	0 (0)	5 (100.0)	0 (0)	0
Seminal fluid	1 (0.9)	1 (100)	0 (0)	0 (0)	0
Blood	9 (7.8)	2 (22.2)	5 (55.6)	2 (22.2)	6
Wound swab	49 (42.2)	3 (6.1)	21 (42.9)	25 (51.0)	14
Urethral swab	3 (2.6)	0 (0)	2 (66.7)	1 (33.3)	2
ECS	7 (6.0)	1 (14.3)	2 (28.6)	4 (57.1)	6
Catheter tip	6 (5.2)	0 (0)	3 (50)	3 (50)	1
Aspirate	5 (4.3)	1 (20)	4 (80)	0 (0)	5
Eye swab	6 (5.2)	0 (0)	3 (50)	3 (50)	5
Total	116	10	65	41	55

HVS-High vaginal swab; ECS-Endocervical swab

Table 3. Antibiotics susceptibility pattern of 116 *S. aureus* strains

Antimicrobial agent (µg)	Number sensitive	% resistant	% intermediate	% sensitive
Tetracyclin (10)	25	78.4	0.0	21.6
Ceftazidime (30)	50	43.9	13.0	43.1
Pefloxacin (5)	80	30.4	0.0	69.0
Vancomycin (30)	116	0.0	0.0	100.0
Linezolid (30)	110	5.2	0.0	94.8
Erythromycin (5)	0	34.8	65.2	0.0
Gentamycin (10)	0	60.9	39.1	0.0
Co-amoxyclav (30)	0	100.0	0.0	0.0

reduced susceptibility to vancomycin of MIC 4 µg/ml and are recommended to be vancomycin intermediate *S. aureus* [9]. Three of the patients were suffering from chronic osteomyelitis while the other two were burn patients, obtained from two of the three hospitals in two states of the region. This reduced susceptibility to vancomycin may be attributed to frequent exposure of the patients to heavy dosage of different antibiotics including vancomycin. The reduced susceptibility to vancomycin is of great concern and threat to public health all over the world; the first reported case was in Japan in 1996 [4]. It has been reported that the presence of a thickened cell wall with several more peptidoglycan layers, compared with non- VISA isolates is the primary factor that causes reduced susceptibility to vancomycin among VISA isolates [11]. Another mechanism reported in USA was identical to that seen in vancomycin-resistant *Enterococcus*. Vancomycin resistant *Enterococcus faecium* harbours the *vanA* operon, which contains five genes [12]. However there has been report of VRSA without *van* gene, this is related to our study where VISA had no *van* gene [13].

Pockets of documented infections due to vancomycin-intermediate *S. aureus* as revealed in this study is a recipe for a full blown development into VRSA if no proper measures are put in place to control the spread. There are certain common features among documented cases of VRSA or VISA [4,5]; the most prominent among which is prior infections with methicillin-resistant *S. aureus* for which patients received repeated and prolonged vancomycin therapy. On the other hand some of the patients had poor clinical response to vancomycin therapy, which is a similar scenario to the five patients with VISA in this study. This finding is a clear signal and warning to development of VRSA in the region where several reports of antimicrobial resistance has emanated for different bacteria [1,14] including MRSA [2]. Moreover, there have been reports of VRSA from the northern part of the country by Olayinka et al. [7] and Onanuga et al. [15] with the prevalence rates of 57.7% and 89.2% at University Teaching Hospital, Zaria and among healthy women in Zaria respectively, including purported 5.4% in fresh and fermented milk from animals [8]. The studies from the northern part of Nigeria based their VRSA assessment on disc susceptibility which is not in line with the recommendation of various international guidelines on antibiotics or vancomycin susceptibility. Also genotypic detection of vancomycin resistance was not included in their respective studies. There is therefore dire need to urgently look into this to ascertain the report coming from this region in view of the possible high level of VRSA from the north and VISA from the south of Nigeria, the most populous black nation in the world is set for serious public health challenge.

5. CONCLUSION

In conclusion, there is a high level of multiple antibiotic resistance in *S. aureus* with some MRSA also showing reduced susceptibility to vancomycin resulting in emergence of VISA. However, the VISA strains have shown no *van* gene as their mechanism of acquiring reduced susceptibility. It is important to prudently recommend vancomycin for treating MRSA or use it in combination therapy. Monitoring for colonization or infection with *S. aureus* with intermediate vancomycin resistance is inevitable among patients who are often treated with vancomycin such as patients on dialysis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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