Molecular Detection of *vanA* Gene in *Staphylococcus aureus* Isolated from Stool Samples in North India

Bassam Y. Khudaier

Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah, IRAQ.

bassamy10@yahoo.com.

http:// DOI 10.29072/basjs.2018204

Abstract

Staphylococci spp. were isolated from 163 stool samples collected from patients in the period from January through August 2007. The isolates included 52 (31.9%) ***Staphylococcus aureus*** *and 111 (68.1%) coagulase negative staphylococci (CoNS) were subjected to minimum inhibitory concentration (MIC) test against vancomycin and teicoplanin. Only four* **S. aureus** *strains were resistant to vancomycin (MIC = 16-64 mg/ml) and teicoplanin (MIC = 32-128 mg/ml). No CoNS isolate exhibited resistance to vancomycin and teicoplanin. Disc diffusion test for vancomycin-resistant* ***Staphylococcus aureus*** *isolates showed high resistance (100%) to ampicillin, ciprofloxacin, erythromycin, oxacillin, penicillin, tetracycline, teicoplanin and vancomycin; they were resistance to clindamycin and streptomycin (75%), and resistance to chloramphenicol and gentamicin (50%). Multiplex PCR assay for the* ***vanA*** *and* ***vanB*** *genes showed that only 4 isolates with the* ***VanA*** *phenotype produced the expected molecular size 1030 bp product corresponding for the* ***vanA*** *gene. None of the remain isolates produced positive results with the* ***vanA*** *or* ***vanB*** *primers.*

Key words: ***Staphylococcus aureus****, stool samples,* ***vanA*** *gene, PCR, India.*

Introduction

The emergence of drug-resistant bacteria is frequently occurs in the intensive care unit (ICU). This is a problem for ICU physicians because there are several pathogens that can only be effectively treated with a limited number of antimicrobial agents, e.g. Methicillin-Resistant *Staphylococcus aureus* (MRSA) [1,2].

Staphylococcus aureus is a major cause of hospital acquired infections, causing high morbidity and mortality world-wide [3,4] The detection of staphylococci with reduced sensitivity to vancomycin is an important issue for clinical laboratories [5]. Interestingly, reduced susceptibility to glycopeptide antibiotics, including vancomycin, has been associated with increased susceptibility to beta-lactams [6,7]. Studies of this phenomenon, termed the "see-saw effect," have produced conflicting clinical reports [8,9].

In 1997, the first strain of *S. aureus* with reduced sensitivity to vancomycin and teicoplanin was reported from Japan [10]. Soon thereafter, a report of two additional cases from the United States was published [11]. There have been considerable research and an array of published reports focusing on this topic during the recent years. The relative high burden of methicillin-resistant *S. aureus* (MRSA) in healthcare and community settings is a major concern worldwide [12]. Report of the national nosocomial infection surveillance (NNIS) system indicated that about 75% of (CoNS) and 47% of *S. aureus* isolates from ICU were resistant to methicillin [13]. Vancomycin remains the drug of choice for treatment of severe MRSA and other infections [12,14].

The objectives of the present study were to screen *S .aureus* and CoNS from stool samples of patients suffering from diarrhea, to determine the antimicrobial sensitivity by disc diffusion test and MIC against various antimicrobials agents, as well as detection of the vancomycin resistant staphylococci spp. by polymerase chain reaction (PCR) amplification of *vanA*, and *vanB* genes.

Materials and Methods

Collection of Samples

In the present prospective study, the screening for staphylococcal spp. was carried out during the period from January to August 2007. The stool samples (163) were collected from

different inpatients and outpatients wards at the Department of Medical Microbiology, Postgraduate Institute for Medical Education Research (PGIMER), Chandigarh, India.

Preparation of Samples and Culturing

All collected stool samples were submitted to the lab in Cary Blair medium (Hi-Media, India), and were inoculated onto blood agar (Difco Laboratories, Detroit, USA) and incubated at 37°C for 18 hrs. Suspected *Staphylococcus* colonies were streaked onto brain heart infusion agar (BHIA) (Hi-Media, India) and incubated at 37°C for 18 hrs [15].

Identification of Species

The identification of isolates of *S. aureus* and CoNS was performed according to standard method described by [15]. Tentative identification for all the isolates was done by traditional culture characteristics, Gram staining, catalase test, coagulase tests and mannitol fermentation tests (Hi-Media, India) [15,16]. Definitive identification up to species level was made with the BD-BBL Crystal Identification System, (Gram-Positive ID Kit-Sparks, Maryland, USA). *S. aureus* ATCC 29213 was used as control strain [17].

Antibiotic Sensitivity Testing

The antibiotic susceptibility profile was determined by the disc diffusion technique, using 12 different antibiotic discs as the following concentrations: ampicillin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), clindamycin (2µg), erythromycin (15µg), gentamicin (10µg), oxacillin (1µg), penicillin (10U), streptomycin (10µg), tetracycline (15 µg), teicoplanin (30µg), and vancomycin (30µg), using Kirby-Bauer method as described by [18] and National Committee for Clinical Laboratory Standards [19]. Mueller-Hinton (MH) agar plates (Difco Laboratories, Detroit, USA) were overlaid with the *S. aureus* clinical strains inoculum (turbidity equivalent to that of a 0.5 McFarland Standard) [16]. Inhibition zone diameters were measured after 24 and then 48 hrs of incubation following [19] criteria. *S. aureus* ATCC 29213 was used as reference strain.

Determination of Minimum Inhibitory Concentration (MIC)

Resistance to vancomycin and teicoplanin were determined by the E-test according to the manufacturer's instructions (AB BIODISK, Solna, Sweden). An inoculum with turbidity equivalent to that of 0.5 McFarland standard. Mueller-Hinton (MH) agar was used. The results were read after incubation at 37°C for 48 hrs. All vancomycin resistant staphylococci

(MICs >6 ug/ml) were also subjected to sensitivity tests by agar dilution method according to the current guidelines of the [14]. Vancomycin resistant *S. aureus* ATCC 29213 was used as quality control strain.

Extraction of DNA

Template DNA was prepared by boiling procedure according to [20].

Detection of Vancomycin Resistance Genes by PCR

To detect genes coding for vancomycin resistance, the specific oligonucleotide primers selected in this study were synthesized by Metabion GmbH, Germany as shown in Table 1 [21].

Table (1): Oligonucleotide primer sequences used for PCR amplification of *van* genes [21]**.**

Primer	Sequence (5'→3')	Gene	Product (bp)
F.1	CATGAATAGAATAAAAGTTGCAATA	*vanA*	1030
R.1	CCCCTTTAACGCTAATACGATCAA		
F.2	GTGACAAACCGGAGGCGAGGA	*vanB*	433
R.2	CCGCCATCCTCCTGCAAAAAA		

The multiplex PCR reaction mixture consisted of PCR Master Mix (Banglore Genei, KT-77) containing 10 mM Tris-HCl buffer pH 9.0, 50 mM KCl, 1.5 mM MgCl2, a total of 1 mM deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 0.5 mM of each primer, 1 U of *Taq* DNA polymerase, 0.01% gelatin and 10 ml purified DNA solution in a total volume of 50 ml. The amplification of DNA was performed on a BIO-RAD thermocycler (CA, USA) that was carried out with the following thermal cycling profile: 3 min at 94°C and 30 cycles of amplification consisting of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C, with 7 min at 72°C for the final extension. The amplicons were analyzed by electrophoresis on agarose gels (1% w/v) (Sigma-Aldrich, Poole, UK) in TAE buffer for 45 min at 100 V. A 100 bp DNA ladder (Bangalore Genei, India) was used as molecular size marker. The gels were stained with ethidium bromide (0.5 mg/ml), visualized under UV transillumination and photographed (Nikon, Japan).

Results and Discussion

The present study showed the staphylococcal isolates from the stool samoles were 163; 52 (31.9%) strains were confirmed as *S. aureus* and the remaining 111 (68.1%) strains as CoNS spp. During the routine screening by slide coagulase test many isolates of *S. aureus* were missed due to their poor sensitivity and thus falsely reported as coagulase negative staphylococci during routine screening process. In the present study, tube coagulase tests of all 163 staphylococcal isolates were performed. Therefore, the main criterion that used for the *S. aureus* identification was tube coagulase test. However, the slide staph plus test was also found to be good parameter to differentiate *S. aureus* from CoNS. Song and his colleagues [22] in 2017, have reported that of the 126 stool samples, 8 (6.3%) were positive for *S. aureus.*

Minimum inhibitory concentration of the 52 *S. aureus* strains and 111 strains of CoNS against vancomycin and teicoplanin have shown that totally four *S. aureus* strains were resistant to vancomycin and teicoplanin (VRSA). Three of *S. aureus* isolates were highly resistant to vancomycin (MIC = 32-64 mg/ml) and teicoplanin (MIC = 32-128 mg/ml), while the other one showed intermediate resistance to vancomycin (MIC = 16 mg/ml) but was resistant to teicoplanin (MIC = 32 mg/ml). No CoNS isolate exhibited resistance to vancomycin and teicoplanin (Table 2).

Table (2): Phenotypes and genotypes frequency of vancomycin resistant *Staphylococcus* spp. isolated from faecal samples.

Species	No. of Isolates (%)		MIC (µg/ml)		Gene Detected	No. of Isolates
			vancomycin	teicoplanin		
S. aureus	3 (1.8)	4 (2.4)	64	128	*vanA*	1
S. aureus			32	32	*vanA*	2
S. aureus	1 (0.6)		16	32	*vanA*	1
S. aureus	48 (29.5)		4	1	-	11
			4	0.5	-	16
			2	1	-	8
			2	0.5	-	13
CoNS	111 (68.1)		4	1	-	19
			4	0.5	-	14
			2	1	-	18
			2	0.5	-	60
Total	163 (100)					163

Vancomycin, a glycopeptide is currently the main antibacterial agent available to treat life-threatening infections with MRSA, including tedizolid, dalbavancin, and oritavancin. [23,24]. The vancomycin resistance among Gram-positive bacteria had been thought to be uncommon in *Enterococcus* spp. [25] [25]. *S. aureus* and CoNS have been reported in various studies [11,26,27,28,29]. Widespread use of vancomycin to treat infections caused by MRSA and other Gram-positive cocci has led to the emergence of vancomycin resistance bacteria [30].

The MICs of vancomycin and teicoplanin against the clinical isolate of *S. aureus* revealed there is an increase in the resistance, 3 of *S. aureus* were highly resistant to vancomycin and teicoplanin (32-64 μg/ml and 32-128 μg/ml), respectively. The remaining *S. aureus* was intermediate resistant to vancomycin (MIC = 16 μg/ml) and highly resistant to teicoplanin (MIC = 32 μg/ml). Assadulla and his team [31] in 2003, have recorded some strains of vancomycin intermediate *S. aureus* (VISA) was isolated in India. Song and his colleagues [32] in 2004, have also been reported the emergence of heterogeneous vancomycin resistant *S. aureus* strains from India and its neighboring countries. Voss and his colleagues in [33] in 2007, have shown that the ability to detect the glycopeptide intermediate *S. aureus* GISA phenotypes is varied significantly between methods and phenotypes. The current vancomycin resistant staphylococci in hospital as well as in community are alarming situation to the clinicians. Another study has reported VRSA strains was recorded in Jordan [34]. This emergence of VRSA/VISA may be due to building of selective pressure of vancomycin.

Disc diffusion test revealed that all vancomycin resistant staphylococcal isolates have shown to be resistant to most of the commonly used antimicrobials (Table 3).

Of greatest concern that the disc diffusion testing is widely used in the United States and around the world [35]. The clinical isolates of *S. aureus* were found to be multidrug resistant to several other antibiotics, they are highly resistant (100%) to ampicillin, ciprofloxacin, erythromycin, oxacillin, penicillin, tetracycline, teicoplanin and vancomycin, and they were (75%) resistant to clindamycin and streptomycin. While they were (50%) resistant to chloramphenicol and gentamicin. The present study is in agreement with the study of Saha and his team [36] in 2008, who demonstrate that ampicillin, ciprofloxacin and erythromycin are 100% resistant to *S. aureus*, while chloramphenicol and gentamicin less resistant.

The 163 *S. aureus* isolates were analyzed genotypically by multiplex PCR with 2 sets of specific oligonucleotide primers for the *vanA*, and *vanB* genes. Only 4 isolates with VanA

phenotype revealed the expected molecular size 1030 bp product corresponding for the *vanA* gene. None of the remaining isolates revealed positive results with the *vanA* or *vanB* primers (Figure 1).

Table (3): Antimicrobial susceptibility of vancomycin-resistant *Staphylococcus aureus* to 12 antibiotics.

Strain No.	Susceptibility to												(%)*
	AM	C	CD	CIP	E	CN	O	P	S	T	TC	V	
1	R	R	R	R	R	S	R	R	R	R	R	R	(91.7)
2	R	R	S	R	R	R	R	R	R	R	R	R	(91.7)
3	R	S	R	R	R	R	R	R	S	R	R	R	(83.3)
4	R	S	R	R	R	S	R	R	R	R	R	R	(83.3)
Total	100	50	75	100	100	50	100	100	75	100	100	100	%

AM, ampicillin (10µg); C, chloramphenicol (30µg); CD, clindamycin (2µg); CIP, ciprofloxacin (5µg); E, erythromycin (15µg); CN, gentamicin (10µg); O, oxacillin (1µg); P, penicillin (10U); S, streptomycin (10µg); T, tetracycline (15µg); TC, teicoplanin (30µg); V, vancomycin (30µg). R, Resistant; S, Sensitive; *, percentage of multidrug resistant antibiotics.

In the present study, PCR assay using DNA of four *S. aureus* phenotypically vancomycin resistant well-characterized genotypically. Strains including vancomycin-sensitive controls as a template confirmed the specificity of the PCR primers (Fig. 1). Each vancomycin resistant enterococci (VRE) exhibited one amplification products with the expected size of 1,030 bp (Table 1) which corresponding to the *vanA* gene resistance genotype of *S. aureus*. No *vanB* gene were demonstrated in *S. aureus* isolates. The remaining isolates of CoNS were repeatedly negative in the assay. There were no discrepancies between the results obtained phenotypically and genotypically by multiplex PCR, may be due to their cell wall thickening is responsible for the development of vancomycin resistance. Hiramatsu and his colleagues [(10)] has suggested that dense accumulation of vancomycin molecules within the thickened cell-wall significantly delays the

timing of complete inhibition of cell-wall synthesis by not allowing efficient penetration of vancomycin molecules through the thickened cell-wall layers [37,38].

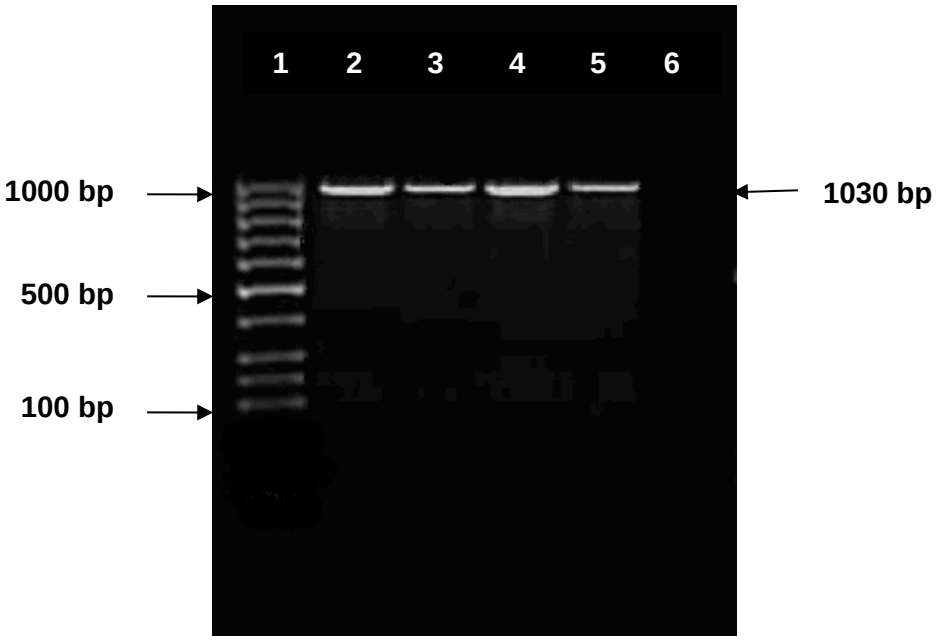

Figure (1): PCR amplification of *vanA* gene. Lane 1, molecular size marker; lanes 2-5, vancomycin-resistant *Staphylococcus aureus* VanA isolates; lane 6, negative control.

Conclusion

From this study we can conclude that *S. aureus* isolated from stool samples showed high resistance to ampicillin, ciprofloxacin, erythromycin, oxacillin, penicillin, tetracycline, teicoplanin and vancomycin. These isolates are harbor *vanA* gene.

The appearance of these highly resistant strains prompt researchers to do a series of studies to assess the colonization and infection with VRSA in patients to define risk factors and to evaluate the effect of interventions on rates of colonization and infection.

References

(1) Kollef, M.H. and Micek, S.T. (2005). Strategies to prevent antimicrobial resistance in the intensive care unit. Crit. Care Med. 33:1845-1853.

(2) Brusselaers, N. Vogelaers, D. and Blot, S. (2011). The rising problem of antimicrobial resistance in the intensive care unit. Ann. Intensive Care. 1: 47-53.

(3) Ben-Ayed, S. Boutiba-Ben, B.I. Boukadida, J. Hammami, S. and Ben-Rdjeb, S. (2010). Hospital acquired outbreak of methicillin-resistant *Staphylococcus aureus* infection initiated by a health care worker. Tunis. Med. 88, 199-202.

(4) De Kraker, M.E. Wolkewitz, M. Davey, P.G. and Grundmann, H. (2011). Clinical impact of antimicrobial resistance in European hospitals: Excess mortality and length of hospital stay related to methicillin-resistant *Staphylococcus aureus* bloodstream infections. Antimicrob. Agents Chemother. 55:1598-605.

(5) Walsh, T. Bolmstrom, A. Qwarnstrom, A. Ho, P. Wootton, M. Howe, R. MacGowan, A. and Diekema, D. (2001). Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J. Clin. Microbiol. 39:2439-2444.

(6) Steinkraus, G. White, R. and Friedrich, L. (2007). Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05. J. Antimicrob. Chemother. 60(4):788–794.

(7) Howe, R.A. Monk, A. Wootton, M. Walsh, T.R. and Enright, M.C. (2004). Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. Emerg. Infect. Dis. 10(5): 855–857.

(8) Ortwine, J.K. Werth, B.J. Sakoulas, G. and Rybak, M.J. (2013). Reduced glycopeptide and lipopeptide susceptibility in *Staphylococcus aureus* and the “seesaw effect”: Taking advantage of the back door left open? Drug Resis. Updat. 16(3): 73–79.

(9) Barber, K.E. Ireland, C.E. Bukavyn, N. and Rybak, M.J. (2014). Observation of “Seesaw Effect” with vancomycin, teicoplanin, daptomycin and ceftaroline in 150 unique MRSA strains. Infect Dis. Ther. 3(1):35-43.

(10) Hiramatsu, K. Aritaka, N. Hanaki, H. Kawasaki, S. Hosoda, Y. Fukuchi, Y. and Kobayashi, I. (1997). Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet. 350: 1670-1673.

(11) Smith, T.L. Pearson, M.L. Wilcox, K.R. Cruz, C. Lancaster, M.V. Robinson-Dunn, B. Tenover, F.C. Zervos, M.J. Band, J.D. White, E. and Jarvis, W.R. (1999). Emergence of vancomycin resistance in *Staphylococcus aureus*. N. Engl. J. Med. 340:493-501.

(12) McGuinness, W.A. Malachowa, N. and Deleo, F.R. (2017). Vancomycin Resistance in *Staphylococcus aureus*. Yale. J. Biol. Med. 90(2): 269-281.

(13) (CDC) Centers for Disease Control and Prevention. (2000). www.cdc.gov/ncidod/hip/ NIS/ DEC2000sar.PDF.

(14) (NCCLS) National Committee for Clinical Laboratory Standards. (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A5. In NCCLS 5th edition. Wayne, PA, USA.

(15) Barrow, G.I. and Feltham, R.K.A. (2003). Cowan and Steel's. Manual for the Identification of Medical Bacteria. 2nd Ed. Cambridge University press Cambridge, London, New York.USA.

(16) Bannerman, T.L. (2003). *Staphylococcus, Micrococcus*, other catalase positive cocci that grow aerobically. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA and Yolken RH. Editor. Manual of Clinical Microbiology. Washington, DC: ASM Press. pp. 384-404.

(17) Macfaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd Ed. Lippincott Williams and Wilkins USA.

(18) Bauer, A.W. Kirby, W.M. Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

(19) CLSI: Clinical and Laboratory Standards Institute. (2014). Performance Standards for Antimicrobial Susceptibility Testing; twenty-first informational supplement. M100- S24, 34:1. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

(20) Reischl, U. Pulz, M. Ehret, W. and Wolf, H. (1994). PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. BioTechniques. 17:844-845.

(21) Clark, N.C. Cooksey, R.C. Hill, B.C. Swenson, J.M. and Tenover, F.C. (1993). Characterization of glycopeptide-resistant enterococci from U. S. hospitals. Antimicob. Agents Chemother. 37: 2311-2317.

(22) Song, X.X. Fang, D.H. Quan, Y.Q. and Feng, D.J. (2017). the pathogenic detection for 126 children with diarrhea and drug sensitivity tests. Eur. Rev. Med. Pharmacol. Sci. 21(4): 95-99.

(23) Binda, E. Marinella, F. and Marcone, G.L. (2014). Old and New Glycopeptide Antibiotics: Action and Resistance. Antibiotics. 3: 572-594.

(24) Rodvold, K.A. and McConeghy, K.W. (2014). Methicillin-Resistant *Staphylococcus aureus* Therapy: Past, Present, and Future. Clin. Infec. Dis. 58(1): S20–S27

(25) Khudaier, B.Y. tewari, R. Shafiani, S. Sharma, M. Emmanuel, R. Sharma, M. and Taneja, N. (2007). Epidemiology and molecular characterization of vancomycin resistant Enterococci isolates in India. Scand. J. Infect. Dis. 39:662-670.

(26) (CDC) Centers for Disease Control and Prevention. (1997). Reduced susceptibility of *Staphylococcus aureus* to vancomycin-Japan, 1996. Morb. Mortal. Wkly. Rep. 46: 624-628.

(27) Ploy, M.C. Gre´laud, C. Martin, C. de Lumley, L. and Denis, F. (1998). First clinical isolate of vancomycin-intermediate *Staphylococcus aureus* in a French hospital. Lancet 351:1212.

(28) Vitali, L.A. Petrelli, D. Lamikanra, A. Prenna, M. and Akinkunmi, E.O. (2014). Diversity of antibiotic resistance genes and staphylococcal cassette chromosome mec elements in faecal isolates of coagulase-negative staphylococci from Nigeria. BMC Microbiol. 14: 106.

(29) Thapaliya, D. Dalman, M. Kadariya, J. Little, K. Mansell, V. and Taha, M.Y. Grenier, D. and Smith, T.C. (2017). Characterization of *Staphylococcus aureus* in Goose Feces from State Parks in Northeast Ohio. Ecohealth. 14(2): 303-309.

(30) Tiwari, H.K. and Sen, M.R. (2006). Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis. 6: 156-161.

(31) Assadullah, S. Kakru, D.K. Thoker, M.A. Bhat, F.A. Hussain, N. and Shah, A. (2003). Emergence of low level vancomycin resistance in MRSA. Indian J. Med. Microbiol. 21: 196-198.

(32) Song, J.H. Hiramatsu, K. Suh, J.Y. Ko, K.S. Ito, T. Kapi, M. Kiem, S. Kim, Y.S. Oh, W.S. Peck, K.R. and Lee, N.Y. (2004). Asian network for surveillance of resistant pathogens study group: Emergence in Asian countries of *Staphylococcus aureus* with reduced susceptibility to vancomycin. Antimicrob. Agents Chemother. 48: 4926-4928.

(33) Voss, A. Mouton, J.W. van Elzakker, E.P. Hendrix, R.G. Goessens, W. Kluytmans, J.A. Krabbe, P.F. de Neeling, H.J. Sloos, J.H. Oztoprak, N. Howe, R.A. and Walsh, T.R. (2007). A multi-center blinded study on the efficiency of phenotypic screening methods to detect glycopeptide intermediately susceptible *Staphylococcus aureus* (GISA) and heterogeneous GISA (h-GISA). Annals Clin. Microbiol. Antimicrobial. 6: 9-13.

(34) Bataineh, H.A. (2006). Resistance of *Staphylococcus aureus* to Vancomycin in Zarqa, Jordan. Pak. J. Med. Sci. 22: 144-148.

(35) Verbist, L. (1993). Relevance of antibiotic susceptibility testing for clinical practice. Eur. J. Clin. Microbiol. Infect. Dis. 12(Suppl. 1): 2-5.

(36) Saha, B. Sing, A.K. Ghosh, A. and Bal, M. (2008). Identification and characterization of a vancomycin resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). J. Med. Microbiol. 57: 72-79.

(37) Cui, L. Murakami, H. Kuwahara-Arai, K. Hanaki, H. and Hiramatsu, K. (2000). Contribution of a thickened cell wall and its gultamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. J. Antimicrob. Chemother. 44: 2276-2285.

(38) Palazzo, I.C.V. Araujo, M.L.C. and Darini, A.L.C. (2005). First report of vancomycin-resistant staphylococci isolated from healthy carriers in Brazil. J. Clin. Microbiol. 43: 179-185.

الكشف الجزيئي عن جين المقاومة للفانكومايسين *vanA* في المكورات العنقودية الذهبية المعزولة من عينات براز المرضى في شمال الهند

بسام ياسين خضير

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة البصرة

البصرة – العراق

bassamy10@yahoo.com

الخلاصة

درست 163 نوع من المكورات العنقودية التي عزلت من براز المرضى من كانون الاول الى اب 2007 . تضمنت الدراسة الحالية 52 (31.9%) عزلة من المكورات العنقودية الذهبية و 111 (68.1%) عزلة من المكورات العنقودية غير المنتجة لانزيم تجلط الدم. درس التركيز المثبط الادنى (MIC) للمضادين الحياتيين الفانكومايسين والتيكوبلانين، اربعة عزلات من المكورات العنقودية الذهبية فقط كانت مقاومة لكلا المضادان الحياتيان الفانكومايسين بتركيز (MIC 16-64 mg/ml) والتيكوبلانين بتركيز (MIC 32-128 mg/ml). لم تبدي المكورات العنقودية غير المنتجة لانزيم تجلط للدم (CoNS) مقاومة لهذين المضادين الحياتيين. أظهرت تجربة مقاومة عزلات المكورات العنقودية الذهبية المقاومة للفانكومايسين مقاومة عالية (100%) لاثني عشر نوعا من المضادات الحياتية بطريقة الاقراص للأمبيسيلين، سيبروفلوكساسين، الاريثروميسين، أوكساسيلين، البنسلين، التتراسكلين، التيكوبلانين وفانكومايسين، واظهرت مقاومة بنسبة (75%) للكليندااميسين والستربتوميسين. في حين كانت 50% مقاومة للكلورامفينيكول والجنتاميسين. أظهرت جميع عزلات للمكورات العنقودية الذهبية المقاومة للفانكومايسين نتيجة موجبة في فحص تفاعل سلسلة البلمرة المتعدد (Multiplex PCR) لجيني المقاومة للفانكومايسين *vanA* و *vanB* حيث أنتجت حزمة بحجم جزيئي 1030 زوج قاعدي للجين *vanA* وقد شكل تطابقا مع النمط المظهري. لم تنتج أي من العزلات الاخرى نتائج إيجابية بالنسبة للجين *vanB*.